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A thirty-year time series analyses identifies coherence between oscillations in Anthrax outbreaks and El Niño in Karnataka, India

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Anthrax is an economically important zoonotic disease affecting both livestock and humans. The disease is caused by a spore forming bacterium, *Bacillus anthracis*, and is considered endemic to the state of Karnataka, India. It is critical to quantify the role of climatic factors in determining the temporal pattern of anthrax outbreaks, so that reliable forecasting models can be developed. These models will aid in establishing public health surveillance and guide strategic vaccination programs, which will reduce the economic loss to farmers, and prevent the spill-over of anthrax from livestock to humans. In this study, correlation and coherence between time series of anthrax outbreaks in livestock (1987–2016) and meteorological variables and Sea Surface Temperature anomalies (SST) were identified using a combination of cross-correlation analyses, spectral analyses (wavelets and empirical mode decomposition) and further quantified using a Bayesian time series regression model accounting for temporal autocorrelation. Monthly numbers of anthrax outbreaks were positively associated with a lagged effect of rainfall and wet day frequency. Long-term periodicity in anthrax outbreaks (approximately 6–8 years) was coherent with the periodicity in SST anomalies and outbreak numbers increased with decrease in SST anomalies. These findings will be useful in planning long-term anthrax prevention and control strategies in Karnataka state of India.

Anthrax is caused by *Bacillus anthracis*, a Gram-positive spore forming bacteria. Outbreaks are most commonly associated with herbivorous livestock (e.g. cattle, buffalo, sheep, goats, and others), although a wide range of animal species, including humans, are susceptible to the disease. Globally, anthrax among animals and people is underestimated and the disease is considered an undervalued zoonosis in terms of the budget spent on prevention and control strategies[1]. In livestock, the disease is usually fatal and outbreaks can result in significant economic loss. Anthrax among livestock presents as an acute or sub-acute infection with a short incubation period of between 36 to 72 hours[2], with prolonged incubation occurring rarely[3]. Humans most often acquire anthrax from infected animals by inadvertently consuming meat or handling meat and by-products such as hides and bones[3–5]. Human anthrax can present in various clinical forms. The two forms of human anthrax most commonly associated through contact with infected animals or animal products are cutaneous anthrax and gastrointestinal anthrax. Cutaneous anthrax is historically considered to be responsible for over 95% of anthrax cases[5], while gastrointestinal infections are less common and associated with the ingestion of infected meat.

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Repeated occurrence of anthrax in a specific geographic region depends on various environmental factors which favour the long-term survival of spores and subsequent uptake of these spores by susceptible host species[6]. Once an outbreak among animals has occurred and resulted in a geographic area becoming contaminated with *B. anthracis* spores, the spores may survive for many years or even decades in the soil[7]. Both the conversion of the vegetative bacteria to spores (sporulation) in the environment and the long-term stability of spores is dependent on the air temperature (9–12 °C), soil temperature, soil *pH* (alkaline pH) and calcium content in the soil[3,5,7]. Previous studies of anthrax in Karnataka found a significant association between livestock outbreaks and environmental variables, such as Land Surface Temperature (LST), Normalized Difference Vegetation Index (NDVI), as well as the presence of various soil components, such as organic carbon, boron, and zinc[8]. In endemic areas, such as the state of Karnataka, these spores may remain dormant in the soil, until certain environmental conditions increase the exposure risk allowing the spores to be more available to susceptible livestock. Though anthrax is considered to be a disease of hot, dry weather, climatic effects may be direct, for example by acting on host susceptibility, or indirect by affecting the availability of nutritious fodder[9,10]. The hot and dry weather may affect the physiological, nutritional status, and metabolic control system of the host, which can modulate the immune response[7], thereby affecting the susceptibility of animals to *B. anthracis*.

In India, anthrax has been reported regularly in various districts within the state of Karnataka (Fig. 1). The office of the Joint Director (Epidemiology) in Karnataka maintains outbreak records, including villages affected with livestock diseases, including anthrax. Currently, vaccination against anthrax is carried out only in affected villages each year using Sterne strain (34F2) vaccine, which provides protection for one year[5]. The vaccination is carried out in the month of January, without consideration of potential seasonality associated with infections. In addition, while there is a system of forecasting anthrax and other livestock diseases in India using logistic regression (www.nivedi.res.in), the current approach forecasts district-level presence or absence two months in advance, but does not account for seasonality or longer-term periodicity that could help in forecasting anthrax outbreaks.

Influences from rainfall, and particularly, rainfall associated with El Niño Southern Oscillation (ENSO) is believed to produce conditions suitable for anthrax outbreaks in Karnataka; however, no quantitative studies have tested this hypothesis. Karnataka state receives 73% of its annual rainfall during the South-West monsoon season (June–September)[11]. There are reports of the influence of ENSO on the Indian monsoon[12–15], including a strong association with North-East monsoon (Oct-Dec months for Karnataka), rainfall extremes over the Southern peninsula of India, and El-Niño[16]. In addition, the impact of the Pacific Ocean El Niño, coupled with

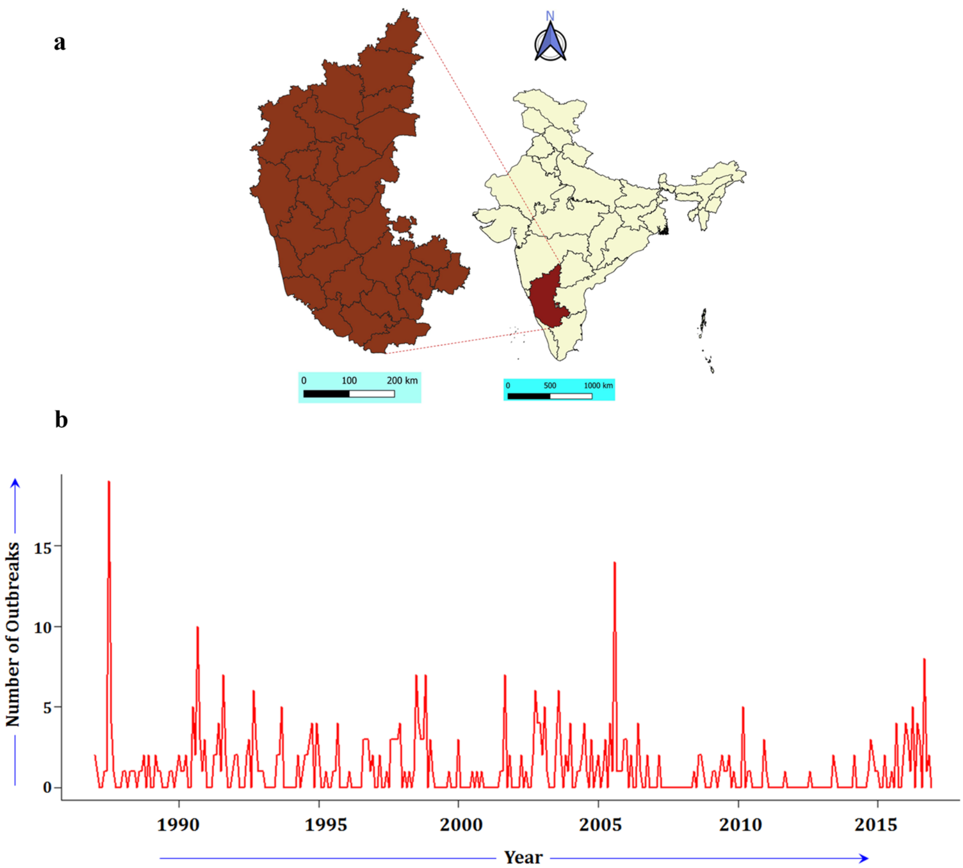

Figure 1. (**A**) Map showing study location Karnataka state in India (Map boundaries are shown as per the guidelines from Survey of India). (**B**) Time series plot of monthly anthrax outbreaks in Karnataka (1987–2016). The x-axis is time in months and y-axis shows the number of the anthrax outbreaks.

the interaction between local air temperatures over land and sea surface temperatures, has previously played a key role in driving rainfall extremes influenced by El Niño in the Southern peninsula of India[16].

Because ENSO is a climatic phenomenon, establishing associations between ENSO and disease occurrence requires analyses of a robust time series data set over a relatively long period[17]. If temporal autocorrelation is not accounted for in a model, it can lead to bias in parameter estimates and can inflate model accuracy[18]. Predicting temporal risk of anthrax is important to implement prevention and control methods for outbreaks among livestock, wildlife, and humans[19]. Specifically, annual livestock vaccination is the primary method of anthrax prevention in India and ideally should be administered to livestock just prior to the onset of expected anthrax outbreaks[20].

Here, we present analyses of thirty-year monthly time series data of anthrax outbreaks in Karnataka, India, using a suite of environmental variables and modelling approaches. The objectives of this study are to characterize periodicity of anthrax outbreaks, meteorological values, and ENSO-related sea surface temperature anomalies; to detect coherence across the study period; and to use a Bayesian predictive modelling framework to further quantify associations between outputs from spectral decomposition methods to advance toward future forecasting of anthrax outbreaks in Karnataka.

Results

A total of 541 outbreaks of anthrax among livestock were reported in Karnataka during the study period, between 1987 to 2016. The maximum number of outbreaks (n = 19) was observed in the month of July, 1987 (Fig. 1) with no visible trend. A cumulative monthly and yearly time series plot of anthrax outbreaks across the study period shows seasonality and inter-annual variation (Fig. S1). The time series plots of rainfall, maximum temperature and wet day frequency shows seasonal pattern (Fig. S2a–c), and SST anomaly plot for the geographical area between 5° N-5° S, 90° -150° W is shown in Fig S2d. The autocorrelation function (ACF) and partial autocorrelation function (PACF) plots of anthrax and rainfall indicated non-stationarity in the time series data, available in Fig. S3 of supplementary information.

Wavelet analyses

Wavelet transformation of individual variables detected strong 12-month periodicity in anthrax outbreaks between 1991 and 1998, but not thereafter; however, significant 6–8 year periodicity (72–96 months) was found throughout the entire anthrax time series (Fig. 2a).

Results from wavelet coherence between anthrax outbreaks and SST anomalies indicated two regions in the time series with significant common power and coherence (Fig. 2b). The first region had strong and coherent 12-month periodicity toward the beginning of the time series between 1991 and 2000. Phase analyses revealed anthrax outbreaks and SST anomaly were in anti-phase with SST anomalies lagging anthrax outbreaks. Additional strong and significant periodicity between anthrax outbreaks and SST anomalies were identified toward the beginning of the time series at 3-year intervals (36 months) from 1992 to 2000, followed by a lengthening in periodicity of 6- to 8-year intervals (72–96 months) from year 2001 to approximately 2009. Phase analysis

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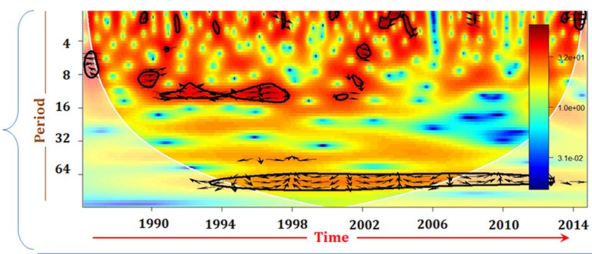

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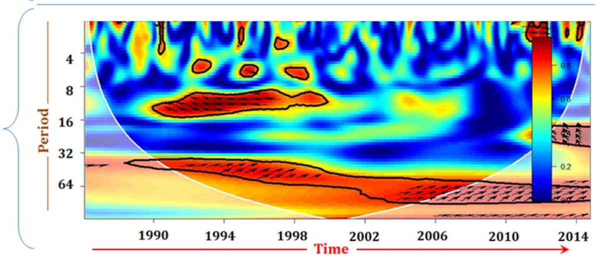

Figure 2. Dominant frequencies in the monthly anthrax outbreaks time series (**a**) and Cross-wavelet coherence between anthrax outbreaks and SST anomalies (**b**) Wavelet power spectrum- The white dotted line is the cone of influence indicating the region of time and frequency where the results are not influenced by the edges of the data and are therefore reliable. The solid black line corresponds to the 95% confidence interval and the areas within this black solid line indicate significant coherence at the corresponding periods and times. Spectrum power in coherence increases from blue to red on the scale of 0 to 1. X-axis: time in months, Y-axis: localised periodicity in months. For Coherence analysis, X variable is SST anomaly and Y variable is anthrax outbreaks.

indicated an opposite pattern observed in the 12-month periodicity; anthrax outbreaks and SST were in phase with SST anomalies preceding anthrax outbreaks.

Bayesian time-series regression model

Results from two sets of Bayesian Poisson generalized linear models (cross-correlated untransformed, pre-whitened variables in the first set and intrinsic model function variables resulting from empirical model decomposition in the second set) revealed interesting shorter-term meteorological and long-term climate and shorter-term association on monthly anthrax outbreaks in Karnataka.

Pre-whitening and cross-correlation function

A lag time is the time between the two-time series for which the cross-correlation is performed. Cross-correlations between the pre-whitened meteorological time series and filtered anthrax time series show significant positive correlations at lag 2 months, and negative correlations at lags 5, 14 and 21 months for rainfall (Fig. 3a). The pre-whitened maximum temperature time series shows a positive correlation with the filtered anthrax time series at lag 5 months and a negative correlation at lag 15 months for maximum temperature (Fig. 3b). Pre-whitened wet day frequency data shows a negative correlation with the filtered anthrax time series data at lag 14 months and a positive correlation at lag 16 (Fig. 3c), and pre-whitened SST demonstrates a small negative correlation with anthrax time series data at lag 15 months (Fig. 3d). A total of twelve variables (rainfall, rainfall at lag 2 and 15 months, maximum temperature at lag 15 months, wet day frequency, wet day frequency at lag 14 and 16 months, SST at lag 15 months and SST anomaly at 15 months), including meteorological variables and their lags, and SST anomalies and their lags were processed for the variable selection procedure.

Empirical mode decomposition

Spectral wavelet analyses provide robust information regarding periodicity in time and frequency domains, but coefficients resulting from continuous wavelet transformations do not necessarily span the entire extent of the study time period (see cone of influence at long periodicities Fig. 2b); therefore, we used empirical mode decomposition (EMD) to extract the seasonal components of anthrax, rainfall, maximum temperature, wet day frequency, and SST anomaly variables. Resulting intrinsic mode functions (IMFs) for each time series served

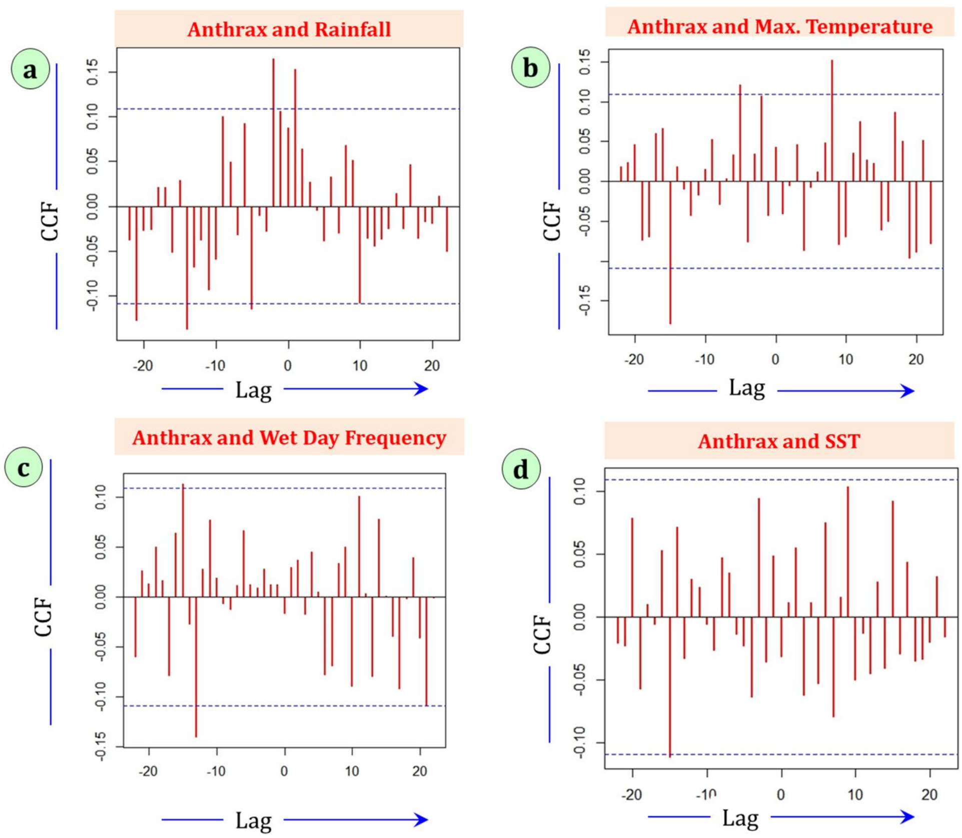

Figure 3. Cross-correlations between with anthrax outbreaks and (**a**) rainfall (**b**) maximum temperature, (**c**) wet day frequency and (**d**) sea surface temperature. The x-axis gives the number of lags in months, and the y-axis gives the value of the correlation between -1 and 1. The blue dashed lines are the 95% confidence intervals for the cross-correlation between two series that are white noise. Identification of significant lag is done by checking the lines beyond the 95% confidence interval on the left hand side of the graph starting from zero lag.

as environmental variables in the Bayesian modelling framework. EMD of anthrax outbreak data are shown in Fig. S8 of supplementary information. The EMD is advantageous over other spectral analysis for analysis of non-stationary time series.

Model selection

Candidate model selection for model sets including untransformed environmental variables and model sets including IMFs of EMD processed environmental variables with monthly anthrax outbreaks as the dependent variable were identified using a genetic algorithm approach, with the best fitting models determined based on the lowest Akaike Information Criterion (AICc) score[21]. The best fitting model for the untransformed environmental variable set included rainfall at lag 2 months, wet day frequency, and SST anomaly. The best fitting model in the IMFs of environmental variables set included IMFs of maximum temperature (IMFs-1 and 5), rainfall (IMF-3), wet day frequency (IMF-6), and SST anomaly (IMFs-2 and 6).

Subsequent Bayesian Poisson generalized linear model analyses identified models including AR (1 and 2) terms accounting for temporal autocorrelation as the best fitting models in both the untransformed and IMF transformed variable model sets, based on lowest Deviance Information Criterion score (DIC) (Tables 1 and 2). Poisson models with AR terms and EMD transformed variables performed better than the model with AR (1&2) terms only (Δ DIC = 10.61). Poisson models with AR (1&2) only performed far better than model with meteorological variables and no AR terms (Δ DIC = 221.49), indicating the importance of accounting for temporal autocorrelation in the modelling process. These results also suggest that a temporally-structured exposure variable missing from our modelling effort may contribute to anthrax outbreak patterns in Karnataka. The Gelman-Rubin diagnostic indicated good mixing and model convergence with values close to 1.0 for each parameter (Table S1), wavelet transformation of model residuals indicated no remaining periodicity, indicating that the model accounted for temporal autocorrelation (Fig S13).

Coefficients for the untransformed variables with AR terms (Table 1) indicated that wet day frequency and rainfall at lag 2 months were positively associated with anthrax outbreaks. The coefficients for the IMFs of environmental variables with the AR terms (Table 2) indicated that IMF-3 of rainfall (annual periodicity) was positively associated with anthrax outbreaks; whereas, IMF-6 of wet day frequency and IMF-6 of SST anomaly, reflective of 8-year periodicity, were negatively associated with anthrax outbreaks. Comparison between the best "untransformed variables" and "IMFs of variables" models shows that the untransformed model performs

Variable	Mean (credible interval) of model with AR (1 & 2) and raw variables (a)	Mean (credible interval) of model without AR (1 & 2) (b)	Mean (credible interval) of model with AR (1 & 2) only and no variables (c)
Intercept	− 0.49 (− 0.84, − 0.16)	0.01 (− 0.08, 0.12)	–
Wet day frequency	0.04 (0.01, 0.07)	0.04 (0.02, 0.05)	–
SST anomaly	0.19 (− 0.06, 0.44)	0.23 (0.14, 0.32)	–
Rainfall at lag 2 months	0.003 (0.001, 0.005)	0.003 (0.002, 0.004)	–
Ar(1)	0.33 (0.13, 0.57)	–	0.41 (0.21, 0.65)
Ar(2)	0.31 (0.04 , 0.55)	–	0.30 (0.05, 0.52)
DIC	927.25	1185.51	939.88

Table 1. Model coefficients, credible intervals and DIC of three models. (a) Model with untransformed variables and autoregressive terms (AR (1 & 2)) (b) Model with only untransformed variables and no AR (1 & 2) terms and (c) Model with only AR(1 & 2) terms and no untransformed variables.

Variable	Mean (credible interval) of model with AR (1 & 2) (a)	Mean (credible interval) of model without AR (1 & 2) (b)	Mean (credible interval) of model with AR (1 & 2) only and no IMF variables (c)
Intercept	− 0.52 (− 0.79, 0.01)	− 0.03 (− 0.14, 0.005)	–
Maximum temperature IMF-1	0.006 (− 0.0008, 0.15)	0.006 (0.001, 0.008)	–
Maximum temperature IMF-5	0.07 (− 0.002, 0.14)	0.07 (0.03, 0.08)	–
Rainfall IMF-3	0.005 (0.002, 0.008)	0.006 (0.004, 0.007)	–
Wet day frequency IMF-6	− 0.11 (− 0.19, − 0.04)	− 0.092 (− 0.12, − 0.082)	–
SST anomaly IMF-2	0.008 (− 0.005, 0.02)	0.014 (0.006, 0.017)	–
SST anomaly IMF-6	− 0.19 (− 0.29, − 0.10)	− 0.14 (− 0.18, − 0.13)	–
Ar(1)	0.31 (0.06, 0.58)	–	0.41 (0.21, 0.65)
Ar(2)	0.12(− 0.14, 0.38)	–	0.30 (0.05, 0.52)
DIC	929.27	1161.37	939.88

Table 2. Model coefficients, credible intervals and DIC of three models. (a) Model with IMFs of environmental variables and AR (1& 2) terms (b) Model with only IMFs of environmental variables and no AR (1& 2) terms and (c) Model with only AR (1& 2) terms and no IMFs of environmental variables.

better than the IMF model; however, the IMF model has lower variance attributable to the autoregressive term of order 2.

A plot of the overall best fitting model, including untransformed variables (rainfall at lag 2, wet day frequency, and SST anomaly) is presented in Fig. 4. A plot of the model with untransformed variables and no AR (1 & 2) is shown in Fig. S9 of supplementary information. Plots of models with IMFs of environmental variables and the AR (1 & 2) term, IMFs of environmental variables only (no AR terms), and an AR (1 & 2) term only model (no meteorological variables, SST, or their IMFs) are presented in Figs. S10, S11 & S12 respectively. Results from out-of-sample prediction indicated that the model with 18 years of test data (1998–2016) and the model with 12 years (2004–2016) of test data both performed better than the model with 5 years (2011–2016) of test data (Fig. S14).

Discussion

Anthrax is known to be a seasonal and periodic disease that naturally occurs in India, and the present study suggests that weather and climatic factors (rainfall, maximum temperature, wet day frequency, and sea surface temperature anomalies) play a significant role in triggering outbreaks among livestock. The present study was geographically focused on Karnataka state in India and used robust mathematical and statistical methods to investigate the associations of anthrax outbreaks among livestock with meteorological variables and ENSO.

Wavelet analysis demonstrates advantages over other spectral methods by identifying the periodicity in both the frequency and time domains[22,23]. Wavelet transformation of the anthrax outbreaks identified significant periodicity around the 6–8-year time period (Fig. 2a). Although, anthrax outbreaks occur among livestock every year, the identification of 6–8-year periodicity indicates that a spike in outbreaks appears to occur at these intervals. This 6–8-year periodicity is significant for planning long-term strategies in prevention and control of anthrax in Karnataka, deploying adequate vaccination, and educating the communities on signs of anthrax and prevention and control methods.

Wavelet coherence analyses between anthrax outbreaks, meteorological variables and SST anomalies was performed in this study to detect coherency between time series pairs in both time and frequency domains. There is a strong coherence between anthrax outbreak occurrence and SST anomaly data, but of varying periodicity (Fig. 2b). Coherence varies from approximately three years during the initial part of the time series and increases to approximately 6–8 years at the end of the series, but the coherence signal is strong and significant (Fig. 2b). The findings suggest an association between ENSO and anthrax outbreaks, as occurrence of ENSO events is also known to occur every of 2–7 years[24]. Coherence between anthrax outbreaks and SST anomaly time series suggests an influence of ENSO on anthrax every 6–8 years and that SST anomaly forecasts can be utilised in making more accurate predictions of anthrax outbreaks among livestock. The influence of El Niño and La Niña produce opposite effects for drought and rainfall respectively, and it varies between countries. The risk of ENSO mediated rainfall and droughts are more commonly studied in Southern Africa and Southern Asia[17]. There are many reports of associations between ENSO and infectious diseases[17,25]. The coherence between ENSO and disease time series vary with the disease and geographical area[17]. For example, analyses of time series of dengue incidence and El Niño were found to be coherent at a 2–3 year periodicity[26], and El Niño at a lag of 12 months was negatively correlated with annual incidence of visceral leishmaniasis, but El Niño at a lag of 36 months had a positive correlation with incidence[27].

Rainfall two months before the occurrence of anthrax outbreaks was positively correlated, but we found a negative correlation at 5 and 14 months before outbreaks with rainfall (Fig. 3a); SST anomalies 15 months before the occurrence of anthrax outbreaks was negatively correlated. Proper prevention and control methods require vaccination of susceptible animals, but there is an additional need for thorough and timely annual vaccination campaigns in endemic districts prior to outbreaks to achieve maximum efficacy and protection of livestock. Timing of vaccination in endemic regions is both a reactionary response and determined by dates of past occurrence

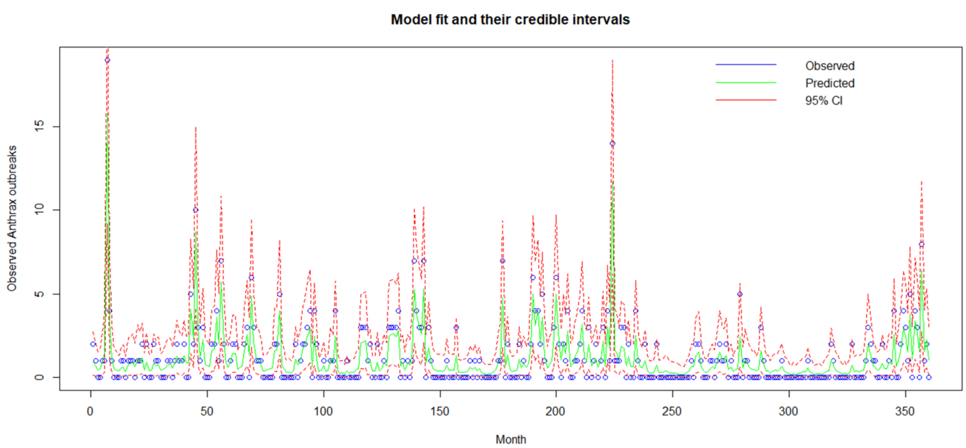

Figure 4. Plot of model fit using selected meteorological variables and the AR (1 & 2) term. Red dotted lines correspond to the 95% credible interval, green solid line is the predicted number of outbreaks and the blue open circles are the observed number outbreaks.

of outbreaks during specific months, but importance of seasonal variation in outbreaks influenced by unusual rainfall or temperature is not considered. The negative correlation of anthrax and rainfall at lags 5 and 14 months (Fig. 3a) may lead to drought-like conditions prompting livestock to graze closer to the soil; a positive correlation between anthrax cases and rainfall two months' prior (lag 2) could unearth the anthrax spores in the soils facilitating consumption by susceptible animals. (Fig. 3a).

Untransformed meteorological variables will have serial correlation, which may hamper accurate detection of associations with anthrax outbreaks. Therefore, in the present study we used EMD for investigating anthrax outbreaks and the association with meteorological variables and SST anomalies. EMD along with wavelet coherence and singular spectral analysis was applied to analyse Buruli ulcer data in French Guiana[28], and the techniques identified significant associations between disease and environmental variables, which was not possible using untransformed data alone. However, there are very few reports on use of EMD processed data as predictor variables using time series models[29].

Bayesian Poisson generalized linear mixed regression model accounting for temporal autocorrelation were used to quantify the association between SST and meteorological variables identified in the cross-correlation analysis with anthrax outbreaks and to quantify associations between the EMD processed variables and anthrax outbreaks. An important finding from our study was a positive association between anthrax outbreaks and wet day frequency, rainfall at lag 2 months and SST anomaly when quantifying associations with the untransformed variables (Table 1), suggesting that seasonality in rainfall is an important predictor of anthrax outbreaks.

Anthrax outbreaks in Karnataka are assumed to occur just after the start of monsoon season (June- September) (Fig. S2a), and hence, our significant finding of a positive association of rainfall 2 months before outbreak occurrence provides quantitative support of these anecdotal reports in the region. Similarly, monthly incidence of human anthrax in China was positively correlated with monthly average temperature, relative humidity and monthly accumulative rainfall with lags of 0–2 months[30]. Similarly, in other endemic countries, outbreaks of anthrax were associated with heavy rainfall[19]. Heavy rainfall is theorized to unearth the *B. anthracis* spores, increasing the risk of contact between spores and susceptible hosts[19]. Meteorological conditions such as drought and breeding stress during the summer seasons may predispose the animals to infection[31]. In other studies, cumulative weather extremes, including prolonged droughts or rains were identified as useful predictors for temporal risk of anthrax epidemics[19].The influence of vegetation indices (NDVI) on mortality due to anthrax in white-tailed deer of Texas, USA was studied and it was found that years with anthrax epizootics were associated with early green springs[32]. The seasonality of anthrax outbreaks is not consistent globally; therefore, a challenge exists in identifying geographically specific climate dynamics contributing to the disease[33]. For example, such geographic variation is suspected to exist throughout India, with reports of a higher number of anthrax outbreaks (38.10%) among animals during the pre-monsoon season in the Indian state of Tamil Nadu during a six-year study period compared to Haemorrhagic Septicaemia (HS), Black Quarter (BQ) and Foot- and- Mouth Disease (FMD)[34]. Identifying a positive association between rainfall two months prior to outbreaks, and a negative association between rainfall 4 and 15 months prior to outbreaks in Karnataka quantifies environmental conditions that can aid in management of decisions specific to this geographic region.

The modelling results in our study using IMFs of meteorological variables and anthrax outbreaks quantified the importance of seasonality and periodicity associated with outbreaks in Karnataka (Table 2). Interestingly, IMF-6 of wet day frequency (representing an 8-year pattern) was negatively associated with anthrax outbreaks and IMF-3 of rainfall (representing a yearly pattern) was positively associated with anthrax outbreaks. This is a similar finding to the long dry spell and higher than normal soil temperature that was observed in epidemiological investigations of an anthrax outbreak in Australia[35]. There was a prolonged period of drying followed by several successive years of flooding before the occurrence of anthrax[36]. However, the importance of drought or flooding may vary between geographic locations[19]. The variability, of either seasonality or periodicity, in the occurrence of anthrax outbreaks may also be influenced by local factors such as regular vaccination of livestock in endemic places, awareness about the disease among farmers, and policies guidelines for proper disposal of infected carcass[37].

The significance of ENSO in determining the periodicity of anthrax outbreaks was supported by the selection of IMF-6, which indicated a 6–8 yearly pattern of SST anomaly in the model. Most notable were results suggesting a negative association with SST anomalies (IMF-6) on the occurrence of anthrax outbreaks; negative SST anomalies indicate the possibility of La Niña—mediated floods. Additionally, there are reports of higher incidence of anthrax associated with movement and occupancy of livestock and humans in the floodplain during dry months resulting in more cases compared to rainy seasons[38].

The time series data can have both seasonality and long-term periodicity which should be properly accounted for, so that an adequate forecasting model can be developed. In our study, we used both meteorological variables (both untransformed and IMFs of EMD) and ENSO data that accounted for temporal autocorrelation in the model. In time series modelling of the data (on both untransformed and IMFs), autoregressive process of order 2 was important in accounting for temporal autocorrelation in model variables and their associations with Anthrax outbreaks (Tables 1 and 2). The autocorrelation should be accounted for in a model using autoregressive terms to prevent bias in parameter estimates[39]. There was both seasonality and significant periodicities in anthrax outbreaks data identified using conventional ACF & PACF plots (Figs. S3a & S3c) and also using wavelet analyses and EMD (Fig. 2a & S6). Seasonality in the outcome variable was accounted for by inclusion of meteorological variables and by autoregressive terms (AR(1) and AR(2)) in the model. However, the autoregressive coefficient, especially the AR(2), dropped significantly in the model with IMFs of meteorological variables compared to the model with autoregressive terms only (Table 2), indicating an influence of long-term variability (periodicity) in meteorological variables and ENSO in predisposing anthrax outbreaks. It also indicates that the seasonality in anthrax outbreaks is dependent on both the AR (1) (anthrax outbreaks one month before) and seasonality in the meteorological variables (for example, rainfall two months before outbreaks). The lagged effect of rainfall and wet day frequency can be employed in short term forecasting of outbreaks.

The findings of this work pose the possibility of aiding long-term contingency measures related to emergency preparedness by considering the possible significance of La Niña on predicting anthrax outbreaks. ENSO events are known to persist for a minimum of 7 months and a maximum of 19 months[40], and this persistence may also affect the occurrence of anthrax outbreaks. The ENSO forecasts[41] may be employed in anthrax forecasting to provide sufficient time for emergency preparedness. The results of the present study have the potential to be used in policy development which can be employed for planning long-term prevention and control strategy for anthrax in Karnataka, and can be extended to other states of India using the present modelling framework. However, as the autoregressive terms explain a large amount of the variability in the outbreaks, there is need to include additional meteorological variables available on license from IMD (Indian Meteorological Department) to improve the model as the "out of fit" forecasts predict the seasonality well (Fig. S14).

It is important to account for temporal autocorrelation in the data to develop forecasting models and is lacking in most of the studies[42,43]. The current forecasting model for anthrax in India is not quantitative prediction but qualitative. Spatial risk map for Karnataka identified high risk areas, but there are no temporal prediction models that can be utilised for early warning of anthrax in Karnataka[44].

There are many studies to show the influence of ENSO events and occurrence of infectious diseases including zoonoses. El Niño influenced the occurrence of RVF in South Africa and Kenya along with other factors[45]. ENSO was also associated with infectious disease hospitalisation, especially vector-borne and enteric diseases in US[46]. The intensity of outbreaks in ENSO connected countries was 2.5–28% higher during El Niño years compared to non- El Niño years[47]. There was increase in leptospirosis by 25% during La Niña and decrease by 17% during El Niño in Colombia[48]. El Niño Southern Oscillation (ENSO) and land surface temperature anomalies were associated with spillover events of bat-borne viral diseases in humans and livestock in Bangladesh and Australia[49]. Transmission risk of zika virus was highest in 2015 since 1950 in South America and it was linked to 2015–2016 El Niño event[50]. The ENSO events may indirectly influence the occurrence of diseases by affecting local weather. There was association between El Niño 4 index and local weather variables and thereby influencing the occurrence and mortality due to rabies in cattle[51]. Further, the risk of and severity of emerging infectious diseases will expand in future climate change scenarios[52].

Establishing the influence of ENSO on infectious diseases requires analyses of both environmental exposure and the role of herd susceptibility in a population. In this study, we used only the occurrence of anthrax outbreaks as no herd immunity data was available. Nevertheless, there was no periodicity in the residuals of the model (Fig. S13) indicating the dominant periodicity in the anthrax time series was accounted for by meteorological and SST anomaly variables. Hence, it can be concluded from our study that short-term rainfall, along with long-term periodicity in SST anomalies (ENSO) are playing dominant roles in driving anthrax outbreaks in Karnataka. In other words, negative deviations in SST anomaly (La Niña mediated floods) precede outbreaks every six to eight years (Fig. 2b) and rainfall plays an important role in the seasonality of anthrax outbreaks. The findings of this study are important in terms of emergency preparedness to prevent future outbreaks based on climate forecasts and timely vaccination.

Limitations of the study

A major limitation of this study is possible reporting bias of anthrax outbreaks. The reporting bias can be due to lack of rapid and sensitive field diagnostics, number of field veterinarians and laboratories in Karnataka state. Improvement in surveillance using sensitive and rapid diagnostic tests will improve reporting of anthrax cases in Karnataka. An additional limitation is the lack of a denominator data in this study. The village is considered as epidemiological unit for reporting outbreaks, but outbreak data were aggregated to the state level. The intensity of the outbreaks (attack rates) would provide epidemiological information that could be incorporated in district level space–time analyses of anthrax data. The anthrax database did not include information on vaccination during the period which is important for using our models in forecasting outbreaks. There was no information on the vaccine coverage. The department must be sensitized to collect the vaccine coverage for anthrax in the state so that it can be analysed by time series intervention models. Furthermore, changing numbers of livestock and breed distributions that may influence the number of anthrax outbreaks were not included in the analyses because the livestock census occurs at a 5-year interval. However, recent study demonstrated that anthrax disease dynamics in mid-latitude grasslands is decoupled from hosts population dynamics and showed importance of environment in determining anthrax outbreak intensity[53]. The other limitation of our study is coarser meteorological data (CRU). Additionally, in our study, the AR (1 & 2) terms explain the greatest variability in the data, and while this may be due to the importance of temporal autocorrelation (past outbreaks), this result may reflect the need to include additional variables; for example, monthly vaccination doses, changes in livestock densities, or other meteorological variables (relative humidity, wind speed, number of dry/wet days etc.) not used in the study. The future studies could benefit by using meteorological data for making accurate predictions of anthrax using the models developed in this study.

Conclusion

Overall, the results presented in this study provide the first quantified evidence of the association between ENSO and anthrax outbreaks among livestock in Karnataka, India. Applying predicted ENSO events may forecast future anthrax outbreaks among livestock in Karnataka and facilitate emergency preparedness and prevention of human disease. With accurate forecasting, anthrax among livestock may be prevented through sufficient procurement of vaccines, well-timed vaccination campaigns and adequate community education. These preventative measures may not only reduce the economic loss associated with livestock disease, but subsequently prevent zoonotic transmission events to the human population. There is also a need to expand this work to other states of India, and possibly to other anthrax endemic countries, to increase our understanding of the ecological dynamics of the disease and influence of extreme climatic events, such as ENSO, so that a global early warning system can be developed to improve both livestock and public health.

Materials and methods
Disease data
Anthrax outbreak data: Anthrax is a notifiable disease in India, and diagnosis of anthrax among livestock is determined using Gram's staining and methylene blue staining to visualize the capsule, known as the M'Fadyean reaction[5]. Identification of suspect cases occurs following sudden death in livestock with oozing of blood from natural orifices. Although not recommended, if the carcass is opened inadvertently, and presence of spleenomegaly is observed, then the carcass is considered as a probable case of anthrax. Confirmation occurs through identification of the etiologic agent, *B. anthracis,* in a blood smear collected from ear piece by microscopy, growth of bacteria in blood agar, and/or subsequent confirmation of *B. anthracis* DNA using polymerase chain reaction (PCR) assays. Recently, PCR assays has been used to confirm the cases of livestock anthrax, but not all of the laboratories in the reporting network have the resources and facility to carry out PCR confirmation. The data set used in the analyses was of confirmed cases of anthrax in livestock (Cattle, sheep & goat), confirmed by both microscopy and culture. District level (admin-2) monthly anthrax outbreak data (1987–2016) were obtained from the NADRES (National Animal Disease Referral Expert System) database of ICAR-NIVEDI (Indian Council of Agricultural Research- National Institute of Veterinary Epidemiology and Disease Informatics), which maintains the livestock diseases database for India and has collated outbreak data every month from different sources since 1987. A village is considered as an epidemiological unit for monthly reporting of anthrax outbreaks. An anthrax outbreak is defined as one or more anthrax cases occurring in a village in a given month. The present dataset is of monthly anthrax outbreaks in all the livestock species for the study period. Although, nationally the data for anthrax is compiled for all the endemic states of India, Karnataka was chosen for this study because the state has a complete time series and has a more reliable reporting system along with other Southern states of India[54]. The district level data were aggregated across Karnataka to calculate the sum of the anthrax outbreaks every month as a state-wide dependent variable. Karnataka has total livestock population of ~ 27 million of which ~ 13 million is bovines (cattle and buffalo), ~ 9 million is sheep, and ~ 4 million is goats (Livestock census 2012).

Meteorological and sea surface temperature data
Temperature and precipitation data

Concurrent monthly maximum temperature, rainfall, and wet day frequency estimates for Karnataka were extracted from the CRU TS4.0 dataset at a 0.5° × 0.5°spatial resolution from the Climatic Research Unit (CRU) (http://www.cru.uea.ac.uk/data), University of East Anglia[55]. All the temperature, wet day frequency and rainfall variables were mean-centred. Centering of predictor variables helps to improve parameter estimation in Bayesian analyses[56] and the intercept is therefore the expected value of the response variable when all the predictor variable values are set to their means. Centering of variables was performed only before using the variables for Bayesian model fitting.

El Niño -3 (sea surface temperature)
There are different ways to measure ENSO, El Niño or La Niña. Air pressure indices like Southern Oscillation index (SOI) is the difference between atmospheric pressure at sea level at Tahiti and at Darwin. During El Niño event, SOI is negative and during La Nina, the SOI index is positive. The Tahiti-Darwin index goes back to the 1800s and was used to relate ENSO and its impact on global climate effects. Sea surface temperature indices like Niño, Niño 2 (Niño 1 + 2), Niño 3 and Niño 4 are used to measure ENSO. Monthly Sea Surface Temperature (SST) (El Niño 3) data from 1987 to 2016 were obtained from the Japan Meteorological Agency (JMA)[57]. El Niño 3 data covers the region between latitudes 5° N to 5° S and longitudes 90° W–150° W[26]. The SST data is used to calculate SST anomalies. The Nino 3 region (5° N to 5° S and longitudes 90° W–150° W) is the region where the SST is observed and any deviations are used to describe an El Niño or La Niña event. Monthly mean of SST is estimated over the Niño 3 region at a 2° × 2° spatial resolution. The monthly SST anomalies are averaged across the Niño 3 region (latitudes 5° N to 5° S and longitudes 90° W–150° W). Based on the five-month running means of monthly SST anomalies, periods are defined which have anomalies of > = ± 0.5 °C. If there is a positive deviation from the five-month running mean, then the month is considered an El Niño month, and if the deviation is negative, then it is known as La Niña. In this study, SST anomaly data (El Niño-3) were used in the analyses.

Statistical methods
To meet the first objective of this study, we used continuous wavelet transformations and wavelet coherence analyses to identify patterns between time series of anthrax cases and environmental variables. To meet the second objective of the study, we generated two sets of Bayesian Poisson generalized linear models for the purpose of prediction of anthrax cases in Karnataka. The first set of candidate models included untransformed environmental time-series data. Cross-correlation functions characterize time lags in the environmental time-series data, and resulting lags served as predictor variables in the model. The second set of candidate models includes environmental time series data, decomposed using empirical model decomposition. Resulting intrinsic mode functions of each environmental time series served as predictor variables. Parameters from best-fitting models in each set were used to predict anthrax cases in Karnataka.

Stationarity

Autocorrelation functions (ACF) and partial autocorrelation functions (PACF) were used to identify whether anthrax outbreaks and meteorological variables were stationary, exhibiting a constant mean and constant variance. Resulting plots were examined to identify significant autocorrelations in each variable at multiple lag distances[58].

Wavelet analysis

Periodicity in the epidemiological time series can be defined as a signal that repeats itself periodically in a time domain[58]. Periodicity in disease data can be due to many factors, including the waxing and waning of immunity in a population, stock replacement of animals, or it can be due to long-term climatic events determining the occurrence of outbreaks[26,59]. Periodicity in the data can be identified using time series techniques in the time and frequency domains[58]. Fourier transformation is the most commonly employed technique to identify periodicity in data in the frequency domain; however, this method is suitable only for stationary data (constant mean and variance)[58]. Therefore, spectral analyses, such as wavelets and EMD, were employed to detect long term periodicity in the non-stationary time series data. Wavelet transformation was first applied to anthrax (Fig. 2a) and SST anomaly data individually to identify the significant periodicity in the variables. Cross-wavelet analysis can also be applied to two-time series simultaneously to identify the dominant periodicities in the time series and coherence in periodicities, using cross-wavelets and wavelet coherence.

Wavelet coherence was performed to identify coherence between anthrax and each meteorological variable, and between anthrax and SST anomalies. Wavelet coherence works on the principle of the phase synchrony concept[60–62], which makes it possible to detect weak coherence in periodicities between two-time series. Wavelet coherence analysis is the preferred approach in non-stationary time series[22,63], as cross-wavelet transformation (matching amplitudes) may not be able to detect weak correlations between two-time series. Wavelet coherence is estimated on a scale from zero to 1.0, similar to the reporting of a correlation coefficient, and it can also be used to identify the localised phase, or relative lag, between the two coherent signals[61]. A wavelet coherence approach is particularly useful when investigating anthrax outbreak data in Karnataka because there is intra- and inter-annual variability in outbreaks. Wavelet analyses were performed using packages *wavelets* and *biwavelet* in R, using the Morlet function as the mother wavelet[64]. Additionally, phase analyses were performed to identify whether SST anomaly, wet day frequency, or maximum temperature preceded or followed anthrax outbreaks.

Bayesian time-series regression model

Two sets of Bayesian regression models were constructed for this analysis. The first set of models included untransformed anthrax outbreak data as the response variable and meteorological variables, SST anomaly, and their significant lags identified using a cross-correlation function (described below) as independent variables. The second set of models included untransformed anthrax outbreak data as the response variable, and empirical model decomposition (EMD) processed intrinsic mode functions (IMFs) of meteorological variables and SST anomaly as independent variables.

Cross-correlation function

A cross-correlation function (CCF) can be used to identify lagged correlations between meteorological variables and anthrax outbreaks. However, when two-time series are serially correlated, performing cross-correlation on an untransformed time series is not advised[58] because the correlation coefficient estimated can be misleading. Hence, pre-whitening is performed when the driving meteorological variables are serially correlated before performing cross-correlation. In pre-whitening, an autoregressive integrated moving average (ARIMA) model was fitted to the driving meteorological variable, and the same model (i.e. with same autoregressive and moving average orders) was used to 'filter' the outcome anthrax time series. The residuals of the driving meteorological and filtered anthrax outcome time series were then used to estimate a correlation coefficient at different lags. The significant lags identified using pre-whitened anthrax outbreaks and maximum temperature, rainfall, wet day frequency, and SST anomaly were used as independent variables in the first out of two Bayesian regression model sets. The cross-correlation analyses were performed using the *tsa* and *forecast* packages in R[64].

Empirical mode decomposition (EMD)

Time series analysis can be performed in time or frequency domain. Presence of periodicity in a time series can be detected by performing time series analysis in frequency domain. Spectral analysis is a time series analysis in frequency domain to identify periodicities in the data. Spectral analysis decomposes a time series into a combination of sinusoids. Spectral analysis can be used for extracting useful information/signal embedded in time series. Fourier transform is most commonly used spectral analysis to decompose time series to extract seasonal signal. Although Fourier transform can extract signal frequency, but it cannot provide information on signal in both time and frequency simultaneously and hence difficult to use in modelling time series data. Empirical mode decomposition is relatively new method that extracts the time and frequency information and can be used for decomposing non-linear and non-stationary time series. The EMD extracts information into a series of Intrinsic Mode Functions (IMFs). The IMFs can be used as predictor variables in time series model to understand the influence of IMFs that are indicative of short-term and long-term periodicity in the data. In the second model set we used intrinsic mode functions derived from empirical mode decomposition (EMD) of the meteorological time series (rainfall, maximum temperature, wet day frequency) data and SST anomaly data. EMD is a technique in spectral analyses to decompose non-stationary time series data. The method is an iterative process to identify intrinsic mode functions (IMFs). A data set is decomposed into a set of IMFs through a sifting process, which obtains the upper and lower envelopes of the time series, computes the mean, and then subtracts the mean from the original times series to generate the first IMF. This process is repeated to obtain additional IMFs; however, at each iteration, the IMF obtained has a longer periodic component, until only a trend signal remains.

In this study, EMD was applied individually to the environmental data and resulting IMFs served as independent variables in the Bayesian regression analysis. EMD analyses were performed using the R package *EMD*[64].

Variable selection

Multi-collinearity for all the meteorological variables, SST anomaly, significant variable lags, and IMFs were tested using a Variance Inflation factor (VIF) analysis[65]. Large VIF values indicate strong collinearity between variables; variables with VIFs > 10 were not included together in candidate models.

Meteorological variables, SST anomaly data, and their corresponding significant lags identified in the cross-correlation analyses were used with a variable selection method to build candidate Bayesian Poisson regression models that included a term to account for temporal autocorrelation. Because of the large number of variables, it would have been computationally prohibitive to explore all possible combinations of meteorological variables identified in the cross-correlation analyses in the model building process using WinBUGS. We used the *glmulti* package in R[66] to automate the initial environmental variable selection process, using a genetic algorithm approach. We used the glmulti function to fit a Poisson generalized linear model with anthrax outbreak data as the response variable and untransformed meteorological and SST anomaly data at significant lags identified in cross-correlation as independent variables. The best model was selected based on the lowest AICc[21]. This method also gives the estimated importance of the variables selected in the top models. It is computed as the sum of the relative evidence weight of all the models in which the particular variable appears[67]. Currently, glmulti does not offer the option to fit candidate models with autoregressive terms. Thus, we chose this approach as a first step, and added AR terms when fitting the full Bayesian model to ensure that we accounted for temporal autocorrelation, reducing the chance for Type 1 errors, and providing a more precise model fit.

The variables identified in this best model were then used as independent variables in a Bayesian Poisson Generalised linear model that included an additional autoregressive term to account for temporal autocorrelation using *R2WinBUGS* package in R[68].

Similarly, IMFs of environmental variables (6 each of rainfall, maximum temperature and wet day frequency) extracted using EMD of meteorological variables and SST anomaly were also first subjected to variable selection using the *glmulti* function before fitting a Poisson Bayesian generalised linear models with temporal autocorrelation in *R2WinBUGS* package in R[68].

Time series model

In the first set of candidate models, association between the monthly numbers of anthrax outbreaks and meteorological variables and SST anomaly were quantified using a Bayesian Poisson generalised linear model with temporal autocorrelation[69]. In the event of finding significant autoregressive structures; AR (1) and AR (2) in the PACF and ACF plots, a Bayesian Poisson model with autoregressive errors was fitted (Eq. 1). To test the influence of autoregressive terms, model with AR (1) and AR (2) without meteorological variables (Eq. 2) and with meteorological variables and AR terms was fitted (Eq. 3).

The number of anthrax outbreaks (y_t) observed in month t with corresponding meteorological variables $X_{1,t}, \ldots, X_{n,t}$ was assumed to follow a Poisson distribution given by

$$\begin{aligned} y_t &\sim Poisson(\mu_t) \\ log(\mu_t) &= \beta_0 + \sum_{k=1}^{n} \beta_k X_{k,t} + \varepsilon_t \\ \varepsilon_t &= \sum_{i=1}^{2} \rho_i \varepsilon_{t-i} + Z_t \\ Z_t &\sim N\left(0,\ \sigma_Z^2\right) \end{aligned} \tag{1}$$

Model without meteorological variables and with AR (1 & 2) (Eq. 2)

$$\begin{aligned} log(\mu_t) &= \beta_0 + \ \varepsilon_t \\ \varepsilon_t &= \sum_{i=2}^{2} \rho_i \varepsilon_{t-i} + Z_t \\ Z_t &\sim N\left(0,\ \sigma_Z^2\right) \end{aligned} \tag{2}$$

Model with meteorological variables only and no autoregressive terms (Eq. 3)

$$\begin{aligned} log(\mu_t) &= \beta_0 + \sum_{k=1}^{n} \beta_k X_{k,t} + \varepsilon_t \\ \varepsilon_t &\sim N\left(0,\ \sigma_e^2\right) \end{aligned} \tag{3}$$

Priors for different parameters.
$\beta \sim N\left(\mu_\beta, {\tau_\beta}^2\right)$—regression coefficients

$$\mu_\beta \sim N(0,0.0001)$$

$$1/{\tau_\beta}^2 \sim Gamma(1,1)$$

$$1/\sigma_e^2 \sim Gamma(1,1)$$

$$1/\sigma_z^2 \sim Gamma(1,1)$$

$\rho \sim Uniform(-1,1)$ -autocorrelation parameter.

Markov Chain Monte Carlo (MCMC) simulations were performed using the Gibbs sampling algorithm implemented in WINBUGS (**W**indows **B**ayesian inference **U**sing **G**ibbs **S**ampling)[70] in the R2WinBUGS package[68]. Two-hundred thousand iterations were performed with two chains, one-hundred thousand burn-in and with 10 thinning. The convergence of the different parameters was tested using Gelman-Rubin statistics and diagnostic plots using the CODA package in R[71]. Relative model performance was assessed using a Deviance Information Criteria (DIC)[72], with the lowest DIC score indicating the best fitting model.

Similarly, a second set of Bayesian Poisson generalised linear models with temporal autocorrelation were constructed with EMD processed IMFs of meteorological variables, SST anomaly and AR(1) and AR(2) components as described above.

A Gelman-Rubin diagnostic statistic was used to evaluate mixing and convergence for each parameter[73]. Temporal autocorrelation was evaluated using a wavelet transformation of model residuals to identify remaining seasonality or periodicity not accounted for by the AR (1 & 2) terms.

Out-of-sample prediction was performed on 2006–2016 (13 years), 2007–2016 (12 years) and 2012–2016 (5 years) anthrax outbreak data withheld from the full time series data set. Parameter values optimized in the best-fitting model were used to predict anthrax outbreaks across the testing data set time series.

Data availability

The environmental data were compiled from third party sources as referenced in the methods. The anthrax data are provided in materials and methods in the form of time series plots and untransformed data is available from the authors on reasonable request, contingent on permission of the Director of ICAR-NIVEDI. The meteorological data and SST data are available and were compiled from third party sources as referenced in the methods.

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Author contributions
MMC and SBS conceived and planned the study, with input from LPC, JS, HW, DH, BVP and SSP. MMC and DH collated and processed anthrax outbreaks data. MMC performed all the analyses and produced output figures. Output figures were edited by SBS. LPC contributed to manuscript construction. All authors contributed to the interpretation of results. MMC wrote the first draft of the paper and all authors contributed to subsequent revisions.

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