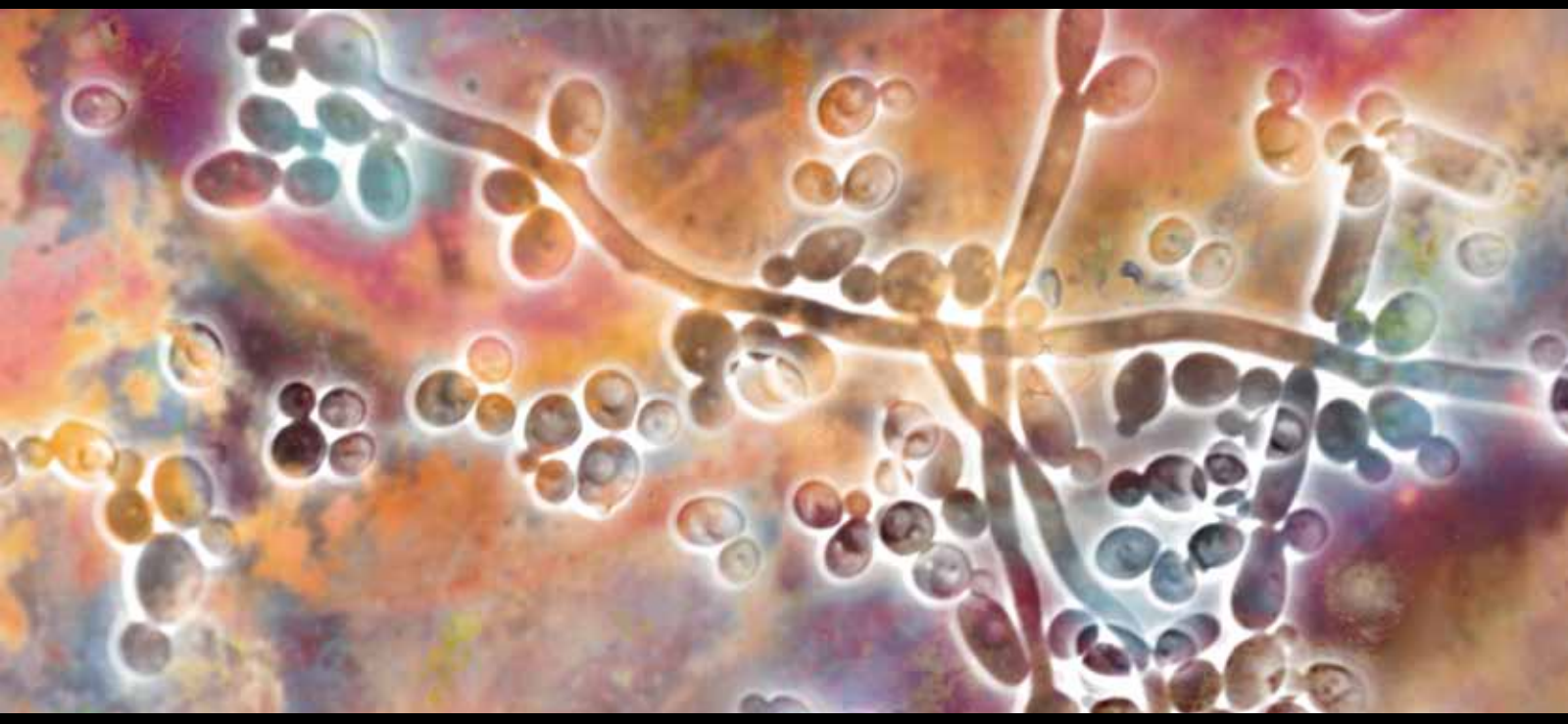
Interdisciplinary Perspectives on Infectious Diseases

Climate Change and Infectious Disease

Guest Editors: Bettina Fries and Jonathan D. Mayer



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Editorial

Climate Change and Infectious Disease

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Due to increasing CO_2 emissions and other greenhouse gases, accelerated global warming is predicted by the Intergovernmental Panel on Climatic Change (IPCC). These climate changes are anticipated to have a long-term impact on marine and terrestrial ecosystems. Rising sea levels are predicted to flood low-lying coastal regions, and fresh water resources could become scarce leading to development of desert-like regions. This will greatly affect the survival of fragile plant species, wild animals, and other ecosystems all of which could directly or indirectly affect survival and or spread of pathogens and their vectors. Although these changes will undoubtedly have an impact on human health, the uncertainties in climate models, as well as predictive epidemiology, hamper accurate predictions on the impact of global warming on public health. In addition, the absence of experimental models that demonstrate dose dependent effects between climate change and disease gives ammunition to those in doubt. As a joint Institute of Medicine/National Academy of Science Committee concluded in 2001, we know that there is a relationship between climate change and infectious disease, but these links are disease specific and location specific, and we cannot yet predict these impacts at anything other than coarse temporal and spatial scales. Thus, the impact on human health is still unclear, and climate change may increase the prevalence of particular infectious diseases in some regions, while decreasing the prevalence in others. There are currently 137 publications in PubMed using the keywords "infectious diseases" and "global warming." The majority of which were published in the last few years, and this attests to the increasing interest in this aspect of public health. However, most data so far are observational, and the predictive understanding of the impact of disease is based on mathematical modeling. Other sources of data include retrospective time-series analyses in specific locations, or use the periodic El Nino Southern Oscillation (ENSO) phenomenon as a surrogate for long-term change. Since controls are usually historical and data are now collected in a more biased fashion than before, there is a concern that some of our conclusions reflect an observational bias that is common when data collection is initiated on new problems that were not previously under rigid observation.

To tackle global warming, coordinated efforts will have to be taken that do not only span countries and cultures but also require many fields of scientific expertise. Effects of global warming are multifactorial and occur at a time when many other important confounding variables constantly change. Beyond global warming, increasing global mobility, genetic modification of agricultural products, and usage of reagents that inhibit pathogens can all have a significant impact on spread and prevalence of infectious diseases. This will make it difficult to establish causality between global warming and impact of infectious diseases. Rigorous scientific conclusions call for experimental models that examine impact of temperature under controlled conditions. Finally, global warming occurs in the general setting of climate change—temperature is not the only variable that is changing. Rather, rainfall patterns, wind patterns, and other climatologic variables are changing at the same time.

As much as we all wish to act fast, and as much as we cry out for an enlightened public consciousness that would be required to press for policy changes that ameliorate the effects of global warming and limit the emission of greenhouse gases, our most imminent problem is that we do not have a true scientific understanding of the matter yet. In this special issue, Interdisciplinary Perspectives on Infectious Diseases solicited cross-cutting interdisciplinary

articles that took new and broad perspectives ranging from what we might learn from previous climate changes on disease spread to integrating evolutionary and ecologic theory with epidemiologic evidence in order to identify key areas for study in order to predict the impact of ongoing climate. These studies present interesting results and analysis of available data. They also highlight how difficult it is to interpret data at present.

Future studies should increase retrospective surveys of museum specimens from previous times, long lasting unbiased data mining and true experimental work on disease manifestation under higher temperatures. Moreover, predictive climate models and predictive epidemiology both need to advance, and become coupled together. This research will likely not meet current output criteria of short-term grant proposals. This problem needs meticulous patient intelligent researches like Darwin to devote their life to answering these pressing questions. Without such efforts, the current generation will inevitably benefit at the cost of generations to come.

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Research Article

Smallpox and Season: Reanalysis of Historical Data

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Seasonal variation in smallpox transmission is one of the most pressing ecological questions and is relevant to bioterrorism preparedness. The present study reanalyzed 7 historical datasets which recorded monthly cases or deaths. In addition to time series analyses of reported data, an estimation and spectral analysis of the effective reproduction number at calendar time t, $R(t)$, were made. Meteorological variables were extracted from a report in India from 1890–1921 and compared with smallpox mortality as well as $R(t)$. Annual cycles of smallpox transmission were clearly shown not only in monthly reports but also in the estimates of $R(t)$. Even short-term epidemic data clearly exhibited an annual peak every January. Both mortality and $R(t)$ revealed significant negative association ($P < .01$) and correlation ($P < .01$), respectively, with humidity. These findings suggest that smallpox transmission greatly varies with season and is most likely enhanced by dry weather.

1. Introduction

Smallpox is the only disease to have been eradicated worldwide [1]. Despite the success story of vaccination and other public health interventions, the number of susceptible individuals has grown to date following cessation of routine vaccination, and the threat of bioterrorist attack has led to debates on countermeasures in such an event [2]. Various mathematical studies have been conducted as part of a preparedness program, including large-scale simulation of a bioterrorist attack and the public health countermeasures against it [3–6]. Theoretical studies on the spread of smallpox include not only simulations but also quantitative analysis of historical data [7–10]. A statistical modeling study suggests that a small outbreak could be contained only implementing contact tracing and isolation [11]. Moreover, those who underwent vaccination in the past are believed to be still protected against severe and fatal manifestations of smallpox even today [7, 12].

Studies on smallpox control have progressed in parallel with the development of epidemiological and statistical methods, and because of the eradication before maturation of biostatistics, many questions have remained in regards to the details of the epidemiology. Seasonal variation in smallpox transmission is one of the most pressing ecological questions playing a key role in determining the transmission dynamics, should a future outbreak occur following the deliberate release [1, 4]. For example, clarification of the seasonal preference of variola virus is crucial for identifying and forecasting the disease risk using ecological data [13]. Although seasonal occurrence of smallpox was documented early on among directly transmitted infectious diseases [14, 15], and whereas the disease is believed to be one of the "winter diseases" in industrialized countries, even the presence of seasonality has not been investigated in detail.

The best available evidence stems from a series of studies by Sir Leonard Rogers (1868–1962) [16], who conducted epidemiologic surveys of smallpox outbreaks in India over a long period of time [17–19]. He also conducted a similar survey in England and Wales [20]. By analyzing the monthly mortality data from the late 19th to the early 20th century in these countries, Rogers argued that the smallpox epidemic in India is relatively uniform (i.e., not apparently cyclical) compared to that in England and Wales [17, 19, 21]. Further, he descriptively and implicitly suggested that there is a negative correlation between humidity and smallpox

mortality, but there was little association between smallpox and rainfall [17, 18]. This effort was followed by Russell and Sundararajan [22] who supported the notion that a dry environment offers favorable conditions for smallpox transmission. These consistent findings have also been reported during the Smallpox Eradication Program (SEP), where a peak of smallpox incidence occurred from December to January in the Northern hemisphere (e.g., Indonesia and Bangladesh) and from May to June in the Southern hemisphere (e.g., Brazil) [23–26]. However, the observed data during the SEP were greatly modified by intensive immunizations, and perhaps because of this, epidemics in other locations were not suggested to be seasonal [26–28], leaving this issue yet to be clarified.

Despite the rigorous efforts before the global eradication, later progress on this issue was unfortunately subtle. Upham once revisited Rogers's dataset from India, anthropologically discussing potential reasons why the American Southwest was less infested by smallpox [29]. A time series technique was applied to historical data in Finland and England [30–32], showing that periodicity is mainly regulated by the susceptible fraction of a population in question [33]. However, despite the analyses on the impact of vaccination and migration on periodicity, seasonal patterns of transmission have not been explicitly studied, mainly because of a lack of data precision. In a historical study examining smallpox in England from the 16th to 17th centuries, the time referred to as the "little ice age," it has been documented that long-term climatic changes did little to the smallpox transmission [34], but this conclusion was drawn without quantitatively and explicitly analyzing the data. Instead, the quality of time series data and its impact on seasonality were discussed in relation to social backgrounds of smallpox control [35, 36], but again no rigorous statistical analyses were made using observed data.

Accordingly, several lingering questions remain. Is smallpox transmission really seasonal? If so, is the seasonality associated with humidity? Clarification of these points will not only enhance our understanding of the pattern of smallpox transmission, but also will be crucial for identifying the seasonal preference of variola virus with some implications for bioterrorism preparedness plans. The present study is aimed at examining the presence of seasonality and clarifying the relationships between smallpox and climate. We reanalyzed various historical datasets, suggesting a new method for the analysis of time series.

2. Materials and Methods

2.1. Data Source: Historical Records. Seven temporal distributions of smallpox at different times and locations were extracted from historical literature. This literature review was based on references collected by tracking all the references given in the relevant publications and repeating this task until we could find no further references; the details are given elsewhere [37, 38]. Figure 1 shows the time series data by location with a monthly reporting interval. Chronologically, epidemic records for The Hague (1755–1773), Berlin (1758–1774), Zurich (1870–1887), the entire Netherlands (1870–1873), Northwest Frontier province in India (1890–1921), Shanghai (1900–1913), and Bombay (1902–1907) provide monthly data of smallpox with time and were used for further analysis [17, 39–45]. The first two records contain data before the introduction of vaccination. Except for Zurich, which documents the monthly number of cases, the remaining datasets record only monthly deaths. Death data are given as the absolute number of deaths, except where indicated. With regard to the magnitude of the epidemics, the annual averages of the disaster size were 10.1 deaths (The Hague), 32.9 deaths (Berlin), 9.9 cases (Zurich), 428.6 deaths (the entire Netherlands), 5.28 deaths per 100 000 (Northwest Frontier province in India), 21.5 deaths (Shanghai), and 2.45 deaths per 100 000 (Bombay). By examining another historical record of the smallpox epidemic in Tokyo, it was found that the mean (and the standard deviation) and the median (25–75% quartile) time from onset to death were 29.1 (13.8) and 26.0 (19.0–37.0) days, respectively [46]. Thus, it is reasonable to assume that the relative frequency of death with time represents that of onset accompanied by approximately a 1 month delay. Moreover, the infection may have happened approximately half a month before the onset [9]. Meteorological variables with time were given only in Rogers's observations [17], which contained the monthly rainfall (inch) and the absolute humidity.

2.2. Time Series Analysis. First, the presence of seasonality was examined for all 7 datasets using spectral density analysis. Spectral analysis is based on the idea of a theoretical power-spectrum, which partitions the total variance of the series among sinusoidal components [47]. In other words, spectral density decomposes a time series function into a sum of sines and cosines. The density plot (i.e., correlogram) was graphically plotted to determine if a sharp peak at a period of 12 months exists, corresponding to an annual cycle (i.e., seasonality).

2.3. Estimation of the Effective Reproduction Number. Second, seasonality that was evaluated using the effective reproduction number, $R(t)$, defined as the actual average number of secondary cases per primary case at calendar time t. $R(t)$ shows a time-dependent variation with a decline in susceptible individuals (intrinsic factors) and with the implementation of control measures (extrinsic factors). If $R(t) < 1$, it suggests that the epidemic is in decline (vice verca, if $R(t) > 1$). This approach was employed to clearly show the seasonal patterns of transmission and to partly address the issue of dependence among cases, that is, statistically, the observation of an infected individual is not independent of other infected individuals, since the disease is transmitted directly from human to human.

The following approximation was made to derive estimates of $R(t)$. Supposing that the number of new infections at calendar time t is $I(t)$, the transmission dynamics are described by the renewal equation using $R(t)$ [48, 49]:

$$I(t) = R(t)\int_0^\infty I(t-\tau)\omega(\tau)d\tau, \quad (1)$$

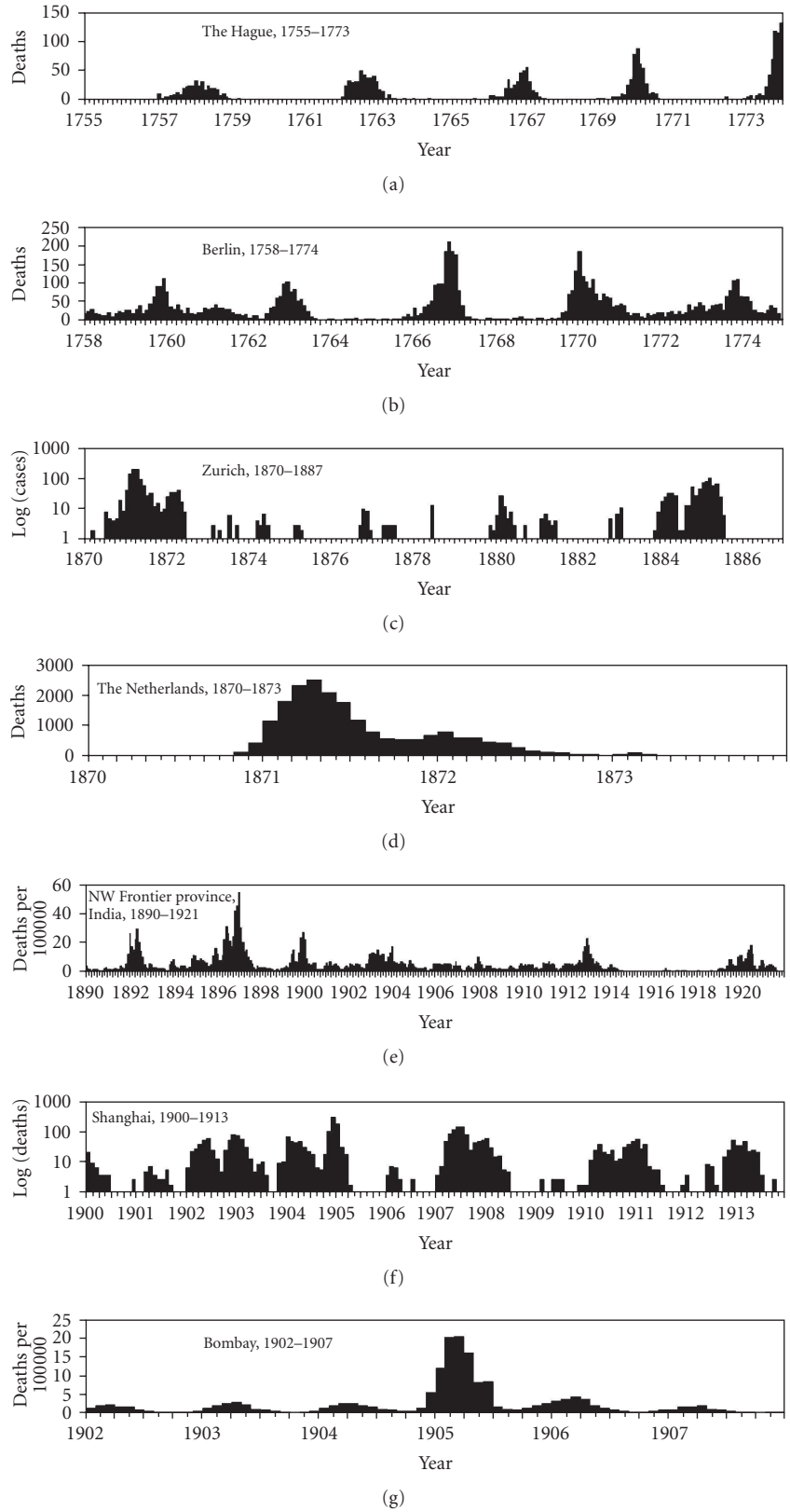

Figure 1: *Temporal distributions of smallpox*. Temporal patterns of smallpox are shown, which were extracted from historical records in (a) The Hague, The Netherlands, 1755–1773, (b) Berlin, Germany, from 1758–1774, (c) Zurich, Switzerland, 1870–1887, (d) the Entire Netherlands, 1870–1873, (e) Northwest Frontier province, India, 1890–1921, (f) Shanghai, China, 1900–1913, and (g) Bombay, India, 1902–1907. Death data are shown in (a), (b), (d), and (f). Cases are shown in (c). Mortality (i.e., deaths per 100 000) data are shown in (e) and (g). See [17, 39–45] for original data.

where $\omega(\tau)$ is the probability density function of the generation time. The right-hand side of (1) represents secondary transmissions at calendar time t, which are determined by the number of those who were infected at time $t-\tau$, $I(t-\tau)$, and the magnitude of secondary transmissions at time t, $R(t)$. Since the data in the present study were recorded only monthly, the equation has to be simplified to comply with discrete points of time data. From the beginning of the history of mathematical modeling of smallpox in the late 19th century [50], cases tended to be modeled by generation, the idea of which is applied as follows. Given the number of cases in generation i, I_i, the expected number of cases in generation $i+1$, $\mathrm{E}(I_{i+1})$ is given by

$$\mathrm{E}(I_{i+1}) = R_i I_i, \tag{2}$$

where R_i is the effective reproduction number of generation i [51]. That is, the reproduction number is simply given by ratio of successive generations of cases, which was implicitly understood in history by a pioneering epidemiologist, Clare Oswald Stallybrass (1881—1951) who applied the theory to analyze the seasonality of various infectious diseases [52, 53]. Since the mean generation time of smallpox is approximately 15 days (i.e., half a month) [50, 54], monthly data contains exactly two generations. Let us consider three successive generations, i, i+1, and i+2. Given the reproduction numbers R_i and R_{i+1}, we get

$$\begin{aligned} \mathrm{E}(I_{i+1}) &= R_i I_i, \\ \mathrm{E}(I_{i+2}) &= R_{i+1} I_{i+1}. \end{aligned} \tag{3}$$

Considering that the generations i and $i+1$ are grouped together and reported in the same month j, the reproduction number cannot be estimated by generation i. Instead, by assuming that the reproduction numbers in the successive generations are identical, that is, $R_i = R_{i+1}(:= R_j)$, (3) can be rearranged as

$$\begin{aligned} \mathrm{E}(I_{i+1}) &= R_j I_i, \\ \mathrm{E}(I_{i+2}) &= R_j I_{i+1}. \end{aligned} \tag{4}$$

The expected number of cases in the next generation $i+3$ is given by product of I_{i+2} and the reproduction number in the next month $j+1$, R_{j+1}, that is,

$$\mathrm{E}(I_{i+3}) = R_{j+1} I_{i+2}. \tag{5}$$

Given (4) and (5), the number of cases in month $j+1$, $C_{j+1}(:= I_{i+2} + I_{i+3})$ is given using $C_j(:= I_i + I_{i+1})$, that is,

$$\begin{aligned} \mathrm{E}(C_{j+1}) &= E(I_{i+2} + I_{i+3}) \\ &= (1 + R_{j+1}) I_{i+2} \\ &= (1 + R_{j+1}) R_j I_{i+1} \\ &= (1 + R_{j+1}) R_j^2 I_i \\ &= (1 + R_{j+1}) R_j^2 \frac{C_j}{1 + R_j}. \end{aligned} \tag{6}$$

We assume that the expected values are sufficient to characterize Poisson distributions. This assumption indicates that the conditional distribution of the reported number of cases in month $j+1$, C_{j+1}, givenC_j is given by

$$C_{j+1} \mid C_j \sim \text{Poisson}\left[\frac{(1 + R_{j+1}) R_j^2}{1 + R_j} C_j\right]. \tag{7}$$

Thus, for the observation of cases (or deaths with a 1 month lag) from month 0 to N, the likelihood of estimating R_j is given by

$$\begin{aligned} L = \text{constant} \times \prod_{j=0}^{N-1} &\left[\frac{(1 + R_{j+1}) R_j^2}{1 + R_j} C_j\right]^{C_{j+1}} \\ &\times \exp\left[-\frac{(1 + R_{j+1}) R_j^2}{1 + R_j} C_j\right]. \end{aligned} \tag{8}$$

By minimizing the negative logarithm of (8), the maximum likelihood estimates of the monthly-approximated reproduction numbers, R_j were obtained.

2.4. Multivariate Modeling. Third, to identify the characteristic factors of seasonal variation in smallpox transmissions, the relationships between meteorological variables (i.e., rainfall and humidity) and incidence (mortality) as well as the effective reproduction number were examined. To examine the influence of seasonal variables on the temporal trend of smallpox, we employed one of the generalized linear models with the construction of a Poisson regression model incorporating monthly and yearly terms [55]:

$$\begin{aligned} \mathrm{E}(C_j) = \exp\Big\{ &\alpha + \beta_1(\text{year}) \\ &+ \beta_2\left[\sin\left(2\pi \times \frac{\text{month}}{12}\right)\right] \\ &+ \beta_3\left[\cos\left(2\pi \times \frac{\text{month}}{12}\right)\right]\Big\}, \end{aligned} \tag{9}$$

where $\mathrm{E}(C_j)$ is the expected number of cases (deaths) in month j, α is a constant, and β_i are the regression coefficients for year or month. The relationship was investigated using both univariate and multivariate models. In the multivariate model, the year of occurrence was controlled for, but the sine and cosine of the month were not included due to colinearity with rainfall. The mortality rate ratios (MRR) for the occurrence of smallpox death were used to evaluate the impact of each meteorological variable on smallpox.

With regard to the relationship between meteorological variables and $R(t)$, multiple linear regression analysis was employed to determine factors contributing to $R(t)$. Because

of the obvious cyclical nature of the observed data yielding an autocorrelation in the linear regression analysis (Durbin-Watson = 0.23), the monthly periodic terms (as shown in (9)) were added to the list of independent variables. The level of statistical significance was set at $\alpha = 0.05$. All statistical data were analyzed using the statistical software JMP version 7.0 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Temporal Distribution and Spectral Density. The spectral densities are shown in Figure 2 which can be reasonably interpreted by comparatively examining the temporal distributions (Figure 1). With regard to the data collected from The Hague and Berlin, the observations of which were made before the introduction of vaccinations, periodic epidemics (i.e., super-annual cycles) are apparent where the interepidemic period ranges from 3 to 5 years (see Figures 1(a) and 1(b)). However, the annual cycle is not seen, and thus, the spectral densities do not show a clear seasonal pattern (Figures 2(a) and 2(b)). On the contrary, the time series data in Zurich and Shanghai clearly revealed a peak at 12 months (Figures 2(c) and 2(f)). The entire Netherlands data covers a relatively short period of time compared to the other datasets (Figure 1(d)) with unclear seasonal and periodic frequencies in the spectral diagram (Figure 2(d)). Although a small peak is seen at 12 months for the data in the Northwest Frontier province in India (Figure 2(e)), the density plot exhibits a multimodal pattern, reflecting an irregular temporal distribution (Figure 1(e)). In the Bombay data, the annual cycle is most clearly highlighted in the temporal distribution (Figure 1(g)), which is also reflected in the spectral density (Figure 2(g)).

3.2. Effective Reproduction Number. Figure 3 plots estimates of the effective reproduction number as a function of calendar time. The vertical broken lines represent January in every year, while a horizontal dashed line is a reference value yielding $R(t) = 1$, that is, the threshold condition of an epidemic. $R(t)$ tends to increase during the winter season for three early records (Figures 3(a), 3(b), and 3(c)), but the annual cycles are not seen. However, the short-term epidemic data for the entire Netherlands clearly shows that three peaks of $R(t)$ coincide in every January with estimates above unity (Figure 3(d)). A similar pattern is observed in Shanghai and Bombay (Figures 3(f) and 3(g)). Figure 4 shows the spectral density plots of $R(t)$ for the entire Netherlands and Northwest Frontier province in India. Although spectral densities of death and mortality (Figures 2(d) and 2(e)) did not exhibit a clear annual cycle, the obvious peak at 12 months is seen for both datasets in terms of $R(t)$ (Figures 4(a) and 4(b)). That is, seasonal patterns of smallpox transmission were reasonably shown with the use of $R(t)$ even for the short- and long-term time series.

3.3. Factors Characterizing Seasonal Variation. Table 1 shows the output of univariate and multivariate models for explaining smallpox mortality in India using meteorological variables. In both models, rainfall was not significantly associated with smallpox mortality. However, significant negative association was found for humidity (adjusted MRR = 0.387 (95% confidence interval (CI): 0.311, 0.481), $P < .01$). Table 2 summarizes the relationship between the effective reproduction number and meteorological variables using a multiple linear regression model. On a whole, the model showed a weak predictive ability. However, humidity was again identified as an explanatory variable which significantly reduces the effective reproduction number ($P < .01$). No significant correlation was found between $R(t)$ and rainfall.

4. Discussion

The present study reanalyzed historical records of smallpox to examine the presence of seasonality and to partly clarify the characteristic factors. Although 18th century data did not show an apparent annual cycle, the remaining records reasonably showed seasonal variations either in the monthly observation or the reproduction number. In particular, even the short-term epidemic data for the entire Netherlands clearly revealed peaks of transmission every January. Although several important meteorological variables were missing (e.g., temperature and atmospheric pressure), Rogers's observation permitted investigations of a few variables as underlying factors characterizing the seasonality. Analyzing the meteorological data in India, both smallpox mortality and the reproduction number yielded significant negative association and correlation with humidity. Rainfall did not appear to be a useful predictor of seasonality.

One important message drawn from this exercise is that smallpox transmission is confirmed as seasonal and this is most likely associated with dry weather. This finding is consistent with implicit suggestions which have accumulated in the historical literature [1, 17, 19]. Whereas the data from The Hague and Berlin did not offer the relevant interpretations, their periodic peaks were also observed during the winter seasons. Assuming that these records captured mainly the large periodic outbreaks alone, it is plausible that the old data were accompanied by under-reporting during less intensive years, and thus, did not precisely contain subtle seasonal fluctuations. Given that the seasonal force of infection was obvious even in the short-term epidemic data from the entire Netherlands, not only endemic but also epidemic smallpox would greatly vary with the season and most likely would be enhanced by dry weather. Historically, virologists attempted to attribute the annual cycle to the seasonal preference of the variola virus [56–58]. To date, it is known that the variola virus could survive in an infective state under different conditions of temperature and humidity [56, 57]. However, as temperature and humidity rise above 30°C and 55%, respectively, the virus is known to immediately lose infectivity [57]. Such a virological explanation supports the epidemiologic findings from this present study and reasonably explains the seasonal preference of the virus as a factor behind the seasonality of outbreaks. The above-mentioned point implies that we

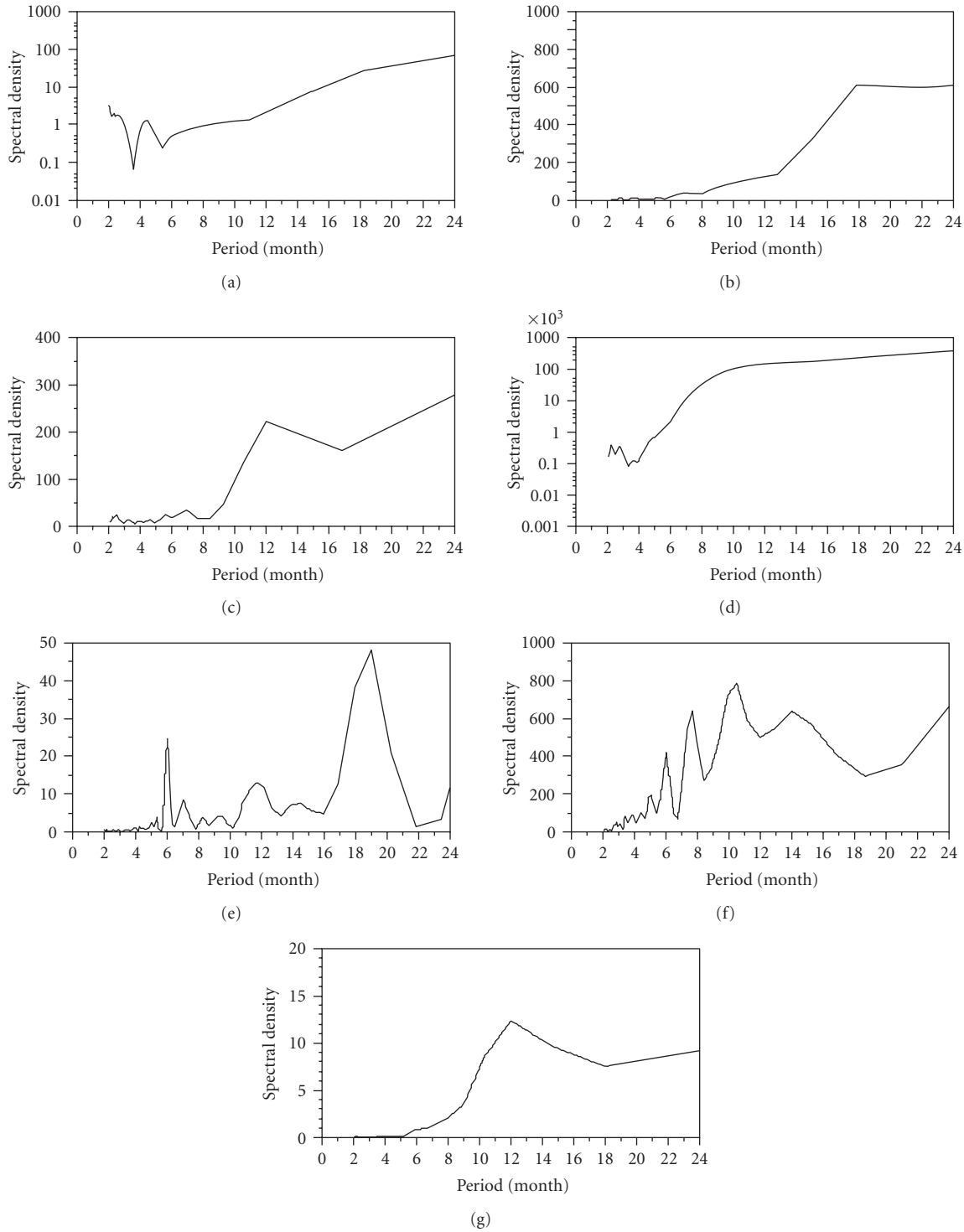

Figure 2: *The spectral density plots for smallpox occurrences.* (a)–(f) correspond to locations as chronologically ordered in Figure 1. A sharp peak at a period of 12 months corresponds to the annual cycle (seasonality), while other longer peaks may reflect a super-annual cycle (periodicity). No adjustment was made in drawing the plots.

cannot ignore the seasonality even in the event of a short-term reintroduction of variola virus due to bioterrorist attack.

A technical development in analyzing seasonal data is also notable. Since the observation of an infected individual is not independent of other infectious individuals, direct application of the generalized linear model has not been appropriate to date. One approach to resolve this issue is to employ a Bayesian method, explicitly dealing with dependence in a Poisson regression model [59], which is,

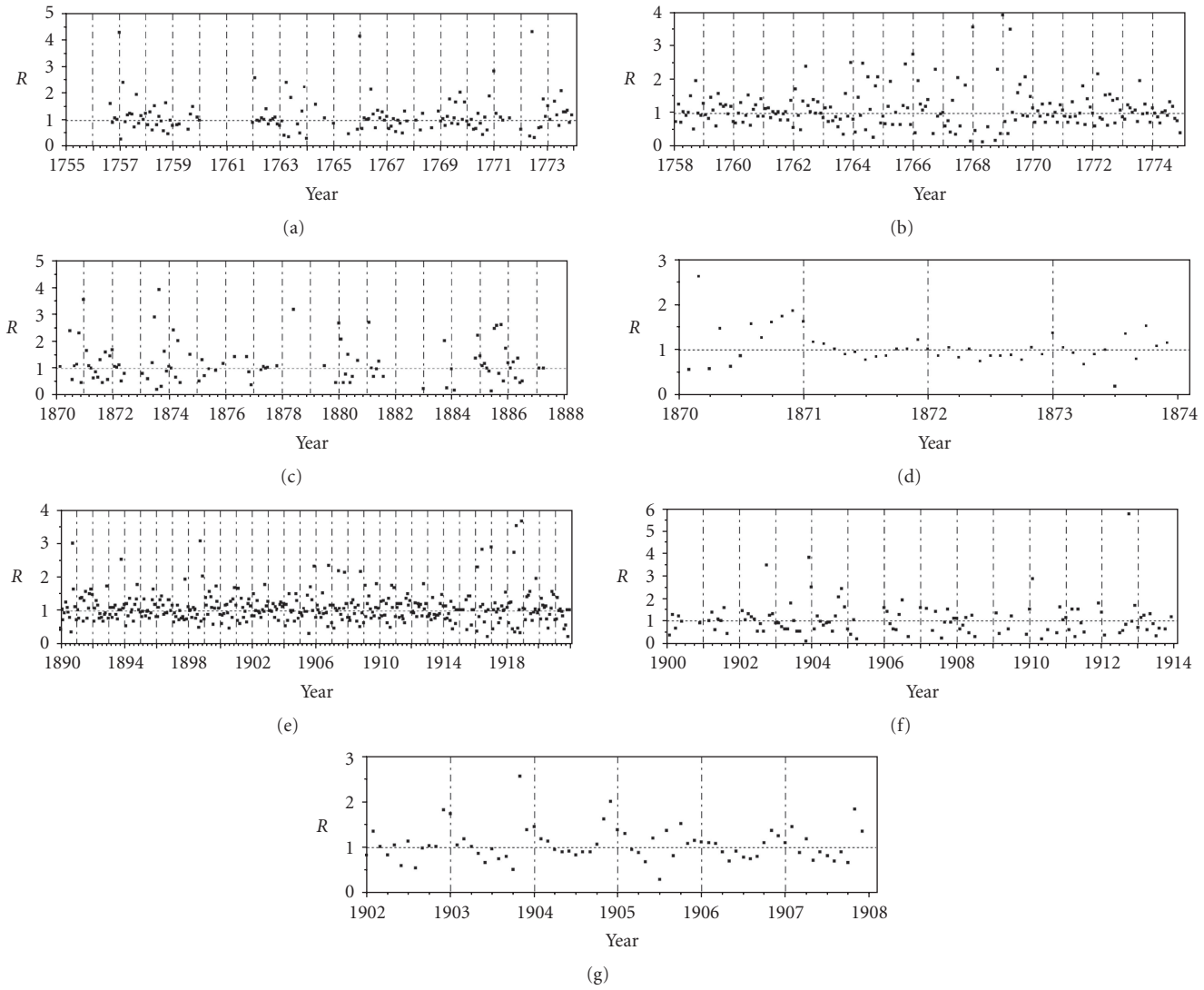

Figure 3: *Estimates of the effective reproduction numbers with time, $R(t)$.* (a)–(f) correspond to locations as chronologically ordered in Figure 1. The horizontal dashed line indicates where the reproduction number is unity. The vertical broken lines represent every January. $R(t)$ cannot be estimated where the observed number of cases (or deaths) was 0 and is not shown for such time points.

Table 1: Monthly weather patterns and smallpox mortality in Northwest frontier province, India, from 1890–1921.

Meteorological element	Univariate model		Multivariate model[¶]	
	MRR[‡](95% CI[†])	*P*	MRR[‡](95% CI[†])	*P*
Rainfall (inch)	0.979 (0.952, 1.003)	.10	0.991 (0.964, 1.018)	.53
Absolute humidity	0.384 (0.309, 0.477)	<.01	0.387 (0.311, 0.481)	<.01

[‡]MRR, mortality rate ratio, reflects change in risk of smallpox death per unit (or absolute value) in the meteorological variable in question. [†]CI, confidence interval. [¶]The multivariate model was also adjusted for the calendar year.

however, computationally complicated for nonspecialists. As an alternative, the present study suggested the use of $R(t)$. $R(t)$ reasonably reflects time-dependent changes in the susceptible fraction of the population in question and other various factors determining the transmission (including seasonality) [60, 61]. In particular, it should be noted that $R(t)$ does not reflect onset or death but can represent an infection event with time, proving its potential as a marker to model seasonal and periodic transmission cycles. In addition, quantitative assessment of $R(t)$ is theoretically important, because the amplitude of seasonal forces of infection characterizes the length of the interepidemic period [33, 62, 63]. A continued super-annual cycle mathematically requires seasonally varying forces of infection, which determines the season-specific threshold condition [64] and evolutionary dynamics of a disease [65, 66]. To the best of our knowledge,

Table 2: Monthly weather patterns and the effective reproduction number of smallpox in Northwest frontier province, India, from 1890–1921.

Variable	$\beta^{\dagger}$	S.E.‡	t-Ratio	P
Intercept	1.579	0.139	11.37	<.01
Rainfall (inch)	0.008	0.015	0.51	.61
Absolute humidity	−1.169	0.319	−3.66	<.01
Cosine	−0.181	0.078	−2.32	.02
Sine	−0.211	0.064	−3.29	<.01

†Parameter coefficient; ‡Standard error; $r^2 = 0.057$ (F-ratio = 5.67, $P < .01$), dependent variable = effective reproduction number, Durbin-Watson = 2.600.

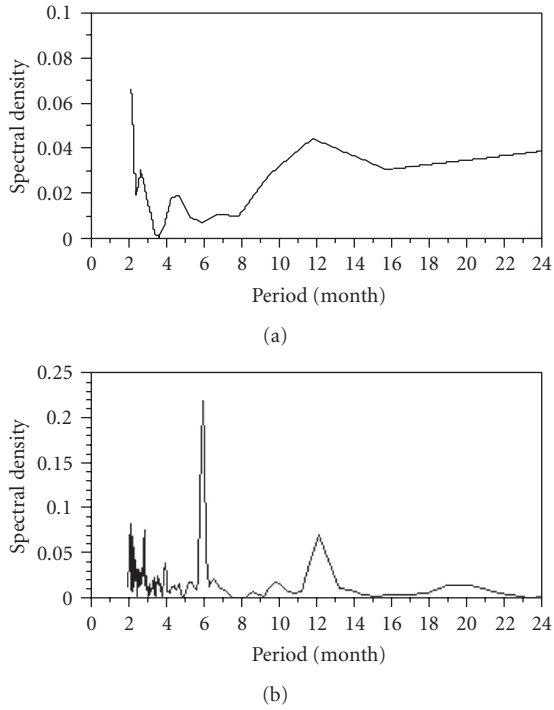

Figure 4: *The spectral density plots for R of smallpox*. The spectral density plots for the estimated reproduction numbers of smallpox in (a) the entire Netherlands, 1870–1873 and (b) Northwest frontier province, India, 1890–1921. Compared with Figures 2(d) and 2(e), the spectral densities clearly indicate a sharp peak at a period of 12 months.

the present study is the first to suggest a quantitative method to reasonably extract the amplitude using the notation of $R(t)$ and extending the previous efforts of Stallybrass [53].

Most infectious diseases show characteristic seasonal variations in incidence. The present study confirms that the transmission of smallpox does vary with season. However, compared to the seasonal ecology of insects in vector-borne diseases, seasonal factors for directly transmitted diseases are far more complex, and thus, questions remain as to what exactly are the factors behind the seasonality of smallpox. At least, experimental evidence supports the role of dry weather in the dynamics of influenza [67, 68]; a recent study found that low (dry) relative humidity in the range of 20 to 30% produced the spread of the influenza virus faster than at relative humidity in higher percentages. In fact, at a humidity of 80% or above, the study found no spread of the flu [68]. Since there are also various social factors which vary with the season, the seasonal preference of pathogens cannot be captured without sufficiently highlighting the time-varying human contact patterns, and thus, further analyses (e.g., reanalysis of small-scale outbreaks where we can adjust the contact frequency) are needed. We hope that the present study enhances the similar reanalysis of historical data, triggering an interest in investigating the relationship between the transmission of directly transmitted infectious diseases and climatic changes.

5. Conclusions

Seven historical datasets of smallpox were reanalyzed to examine the presence of seasonality and to identify the characteristic factors. Annual cycles were clearly shown not only in the monthly reports but also in the estimates of the effective reproduction number. Even for a short-term epidemic, the transmission of smallpox would most likely be enhanced by dry weather.

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Review Article

Effects of Climate Change on Ticks and Tick-Borne Diseases in Europe

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Zoonotic tick-borne diseases are an increasing health burden in Europe and there is speculation that this is partly due to climate change affecting vector biology and disease transmission. Data on the vector tick *Ixodes ricinus* suggest that an extension of its northern and altitude range has been accompanied by an increased prevalence of tick-borne encephalitis. Climate change may also be partly responsible for the change in distribution of *Dermacentor reticulatus*. Increased winter activity of *I. ricinus* is probably due to warmer winters and a retrospective study suggests that hotter summers will change the dynamics and pattern of seasonal activity, resulting in the bulk of the tick population becoming active in the latter part of the year. Climate suitability models predict that eight important tick species are likely to establish more northern permanent populations in a climate-warming scenario. However, the complex ecology and epidemiology of such tick-borne diseases as Lyme borreliosis and tick-borne encephalitis make it difficult to implicate climate change as the main cause of their increasing prevalence. Climate change models are required that take account of the dynamic biological processes involved in vector abundance and pathogen transmission in order to predict future tick-borne disease scenarios.

1. Introduction

Zoonotic tick-borne diseases in Europe have become increasingly prominent since the emergence of Lyme borreliosis (LB) in the early 1980s, and the incidence of this disease and that of tick-borne encephalitis (TBE) have risen dramatically over the last two decades [1]. Both diseases are transmitted by hard ticks of the *Ixodes ricinus* species complex (*I. ricinus* and *I. persulcatus)* and since these ticks spend most of their time in the environment, climate change is likely to affect their distribution and abundance and, therefore, the incidence of disease. *I. ricinus* and *I. persulcatus* are particularly sensitive to environmental conditions since in their prolonged nonparasitic phases they require a microclimatic relative humidity of at least 80% to avoid fatal desiccation. They are, therefore, restricted to areas of moderate-to-high rainfall where there is a good cover of vegetation, so that the soil surface remains humid through the driest times of the year [2]. The Fourth Assessment Report of the Intergovernmental Panel on Climate Change [3] reported that in northern temperate Europe temperature increases of 1.5–2.5°C may occur over the next few decades as a result of global warming. Such climate change may extend or curtail host-seeking tick activity periods, potentially increasing or decreasing tick abundance and distribution, and effects on tick development rates can change seasonal activity patterns by altering the proportion of the tick population that are exposed to regulatory mechanisms such as diapause. In areas where lowered summer precipitation coincides with raised summer temperatures, the survival, activity, and distribution of *I. ricinus* and *I. persulcatus* are likely to be reduced because of their vulnerability to desiccation. These tick species acquire their hosts by ambushing them from the vegetation and a significant number of large animals, such

as deer, must be present in the habitat in order to feed the adult females and thus maintain the tick populations. The more catholic host preferences of the immature tick stages (larvae and nymphs), which parasitize reptiles, small and medium-sized mammals and birds, in addition to large mammals, contribute significantly to the circulation of diverse pathogens between the tick and host populations. Climate change may, therefore, exert a major influence on both tick abundance and disease prevalence by affecting faunal diversity [4].

Other important European tick species such as *Rhipicephalus sanguineus* and *Dermacentor reticulatus* can be affected by climate change through similar mechanisms, but anthropogenic factors also have profound effects on disease incidence, and separating these from the influence of climate change represents a major challenge. In this review attention is mainly focused on evidence for the effects of climate change on the distribution and abundance of European ticks. The potential impacts of climate-change effects on the incidences of the diseases they transmit are discussed.

2. Effects of Climate Change on Tick Distribution and Abundance

2.1. Ixodes ricinus. The climate is regarded as the principal restricting factor at the northern limit of *I. ricinus* distribution [5]. Although *I. ricinus* is surprisingly cold-hardy and when winter-acclimatized can survive 24-hour exposure to temperatures ranging from −14.4°C to −18.9°C [6], the detrimental effects of cold are accumulative and exposure for 30 days to only −10°C has been shown to be lethal for a high proportion of unfed nymphs and diapausing engorged larvae and nymphs [6]. Molting *I. ricinus* ticks are even more vulnerable so that if summer temperatures are not high enough to complete development before the onset of winter (little or no development takes place between 7 and 10°C [7, 8]), they are unlikely to survive even moderate frosts. Degree-day models have been developed which proved useful in elucidating the *I. ricinus* life cycle [9, 10], but more research on the winter biology of this species would help further understand its northern distribution limit.

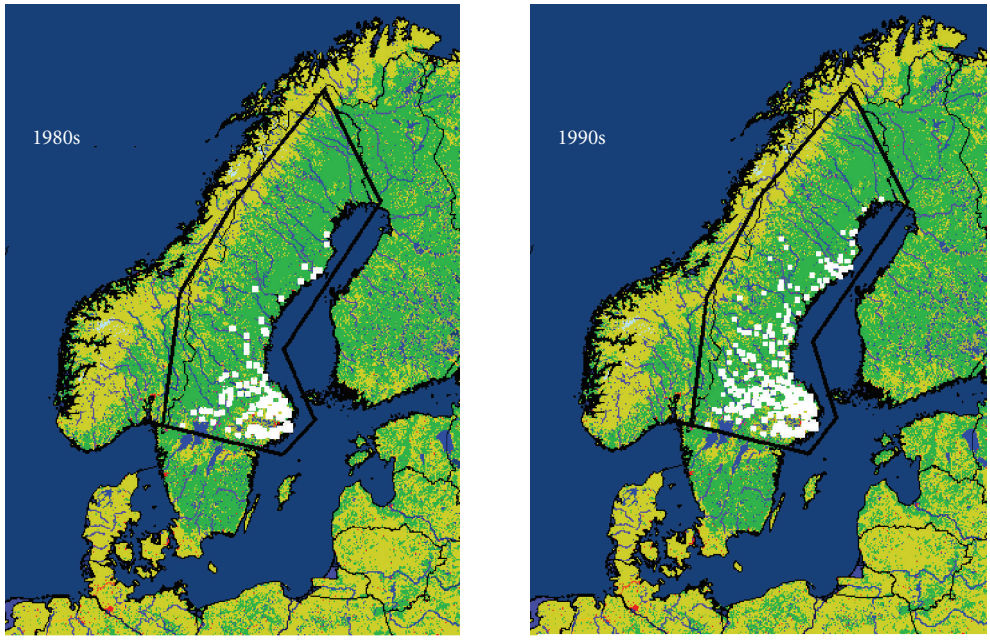
In Sweden, the northern distribution limit of *I. ricinus*, together with that of several other animal and plant species, has shifted northwards since the climate started to noticeably change in the late 1980s [11]. The geographical distribution range of *I. ricinus* used to be located below 61°N [5], but ticks are now established along the whole Baltic Sea coastline (up to 66°N) and along the river valleys and the larger lakes in the northern regions. This shift in latitude distribution has been shown to be related to changes over several seasons in the number of degree-days with temperatures vital for tick survival, activity and development [12]. At the highest latitudes fewer days with cold winter temperatures (well below −12°C for longer periods) had the clearest impact for new tick establishment (Figure 1).

In central Sweden (59°N to 61°N) in areas with medium-high densities of ticks, increases in tick abundance were correlated to a combination of mild winters (fewer days with temperatures below 7°C) and extended spring and autumn seasons (more days with minimum temperatures not lower than 5 to 8°C). In south and central Sweden, the current climate only allows a tick activity season of 6–8 months compared to as much as 11 months in some parts of the British Isles, and further changes in seasonal climate in Sweden are likely to continue to have a major impact on the prevalence of ticks. The combination of climate variables affecting ticks has also been shown to be significantly correlated with increases in the incidence of TBE in Stockholm County (59.2°N) during the period 1960–1998 [13]. The risks of LB are likely to increase as well because *Borrelia burgdorferi* sensu lato (s.l.) spirochetes have been found in *I. ricinus* throughout its range in Sweden [14].

A comparable situation has been reported in changes to the altitude distribution of *I. ricinus* in the mountainous regions of the Czech Republic. Field studies in 1957 and in 1979-80 showed that ticks were prevalent up to 700 meters above sea level. Ticks were collected from dogs or by drag-sampling in the same locations in 2001 and 2002 and were then found as high as 1100 meters in areas where they had been absent from small mammal samples [15–17] and where it has been shown that they could not complete their life cycle over the period 1957–1983 [18]. Furthermore, the prevalence of ticks carrying the TBE virus or *B. burgdorferi* s. l. spirochetes also seems to have increased at high altitude in the Czech Republic [16, 19].

2.2. Dermacentor reticulatus. Dermacentor reticulatus is a vector of canine babesiosis, tularemia, Q-fever, and at least one zoonotic rickettsiosis [20] and has vector competence for *Anaplasma marginale* [21]. Unlike *I. ricinus*, only the adults quest for hosts on surface vegetation and they feed primarily on deer, frequently bite dogs, but only occasionally humans, whereas the larvae and nymphs parasitize rodents. The life cycle is much shorter than that of *I. ricinus*, with eggs deposited in the spring and developing to adults within the same year [22]. The geographic range of the species extends from France and southwest England in the west to central Asia in the east. In western and central Europe its northern limit is northern Germany, northern Poland, and Lithuania, and its southern limit is the Mediterranean shore (restricted to humid mountainous areas), whereas it has a more northern distribution in the east (St. Petersburg). Within this area, its distribution is highly focal, and within Germany in 1976, it was only reported from four sites out of more than 3000 [23].

In two recent studies, however, data were collected showing that this tick species has since colonized many more sites in Germany [19]. The first study (2003) consisted of the screening of 365 dogs from 171 sites. Almost 10% of the ticks from 41 dogs were *D. reticulatus* and the infested dogs came from 26 sites, all previously unknown for the tick. Seven of the sites were subsequently confirmed by flagging. In the second study (2004), 721 deer were shot at 201 sites from 161 districts and their heads examined for ticks. A total of 23 (3.2%) deer from 14 sites were infested and only two sites were already known for *D. reticulatus.* These results



(a) (b)

Figure 1: Changes in tick distribution in northern and central Sweden. White dots illustrate districts in Sweden where ticks were reported to be present before 1980 (a) and in 1994-1995 (b). The study region is within the black line (Lindgren et al. 2000, [12] with permission from *Environmental Health Perspectives*).

strongly suggest that *D. reticulatus* has expanded its range within the last three decades particularly in the eastern and southwestern parts of Germany. Further evidence for the changing distribution of *D. reticulatus* is provided by the occurrence of canine babesiosis in new areas of Germany [24, 25], Hungary [26], Switzerland [27], and the Netherlands [28].

Several factors, perhaps acting synergistically, could be responsible for this recent spread of *D. reticulatus*, including increased deer abundance and the availability of more fallow land as a result of EU agricultural policies. However, there are good reasons for implicating a warming climate as being at least partly involved. Habitats with adult *D. reticulatus* are all characterized by more or less intense solar radiation and it is likely that the temperature sum (cumulative day-degrees above the developmental zero within one vegetation period) at the soil surface is a limiting factor for oviposition and embryonic development [20]. The fact that *D. reticulatus* occurs further north in eastern Europe supports this view, since the continental climate there is characterized by higher summer temperatures. Whereas adults are cold-hardy and can survive continental winters [29], eggs and larvae that do not complete development would not have survived the cold season of a few decades ago in central Europe [30]. Further research to clarify the situation could focus on suitable habitats in the north of Germany.

2.3. Hyalomma marginatum. *H. marginatum* is well known as a vector of the dangerous viral zoonosis Crimean-Congo hemorrhagic fever (CCHF) [31]. Its possible northern spread and establishment of permanent populations is thus of much significance, especially since immature stages are frequently found on migratory birds flying to temperate Europe [32]. The life cycle of this tick is faster in southern parts of its distribution range (Northern Africa) [32] with larvae active as early as February, but clearly slower in northern, colder regions, with immatures active as late as June. Analysis of the recorded distribution of the tick show that, according to climate requirements, there are two clear clusters of populations [33]. One cluster extends from the northern geographical limit of the species in the Balkans (approx., latitude 44°N) and into Turkey and the Middle East. The second one is restricted to Africa north of the Sahara and western parts of Spain. Analysis of the climate niche of the first cluster clearly points to a temperature-related limiting factor for these northern populations. Temperatures between September and December are critical for the establishment of permanent populations. Cumulative temperatures between September and December have an average of 800°C in places where the tick has permanent populations, and below 400°C in sites not colonized by *H. marginatum*. This finding seems to be related to the factors that affect molting of immature stages and are not connected to the extremely cold winter temperatures that prevent overwintering adults surviving into the next year, as suggested by Hoogstraal et al. [32]. If temperatures are high enough to allow molting before the cold winters, unfed adults can survive the next active season. Field observations (Z. Vatansever, personal comment) recorded the feeding of nymphs in late summer in Turkey, with the resulting unfed flat adults commonly

overwintering in the first few centimeters below the soil surface. Regulatory variables for these northern populations appear to act on thermodependent phases of the tick life cycle. On the other hand, climate niche analysis of the southern cluster of tick communities points to a strict dependence on rainfall and potential evaporation, but this may not be relevant if specimens from the southern range can adapt to the colder conditions of the northern cluster.

Although migratory birds are carriers of immature *Hyalomma* ticks and could potentially introduce them into currently *Hyalomma*-free areas in the spring, their climate requirements and current climate data do not suggest that they can become established. Mid-March and early April are the main periods of mass arrival of birds in Spain on their way to northern Europe. Data obtained from the Climate Research Unit (UK) show that average temperature in that period is 16-17°C in Morocco and Mauritania, 9–11°C in southern Spain, and 5-6°C in southern Germany. According to data on molting of engorged nymphs under laboratory conditions, about 300ºC cumulative degrees above the developmental zero (14–16°C) are necessary to complete the molt [34]. Nymphs that engorge at the time of migratory bird arrival in early spring would need much longer to molt in southern Germany, with a consequent increase in mortality, than in north-western Africa, where only a few weeks are required. In the current climatic conditions, it is highly improbable that engorged nymphs can survive in sufficient numbers to be founders of new permanent populations in Europe. Immature *H. marginatum* are found on local (non-migratory) birds in central Spain around late May and early June, which is too late for northern African and southern European populations of *H. marginatum* to mix because of the current climate barriers imposed by their respective climate requirements at the moment of bird migration. If climate change includes the predicted temperature increases, *H. marginatum* ticks may become established in northern latitudes but it is debatable whether initial introduction will occur as a result of bird migration alone because very small numbers of ticks, all immature stages, would be involved. It is more likely that, as autumn and winter temperatures rise, establishment of *H. marginatum* will mainly result from the introduction of adult females feeding on wild and domestic ruminants via the Middle East and the Balkans, where there is much uncontrolled movement of livestock.

2.4. Rhipicephalus sanguineus. A special case of distribution and association to environmental variables is that of the brown dog tick, *Rhipicephalus sanguineus*. It has a worldwide distribution mainly because of introduction by dogs, but rarely occurs in temperate and cold regions. However, *R. sanguineus* is an endophilous tick (associated with shelters like kennels, private gardens, or cracks in walls of human constructions), so may potentially cause temporary infestations in heated accommodation anywhere in the world. Within its normal range, *R. sanguineus* can reach huge populations under adequate environmental conditions and continued presence of a blood source. Currently, the brown dog tick is extremely common around the Mediterranean region. In the coldest places of this region, the tick may undergo a winter dormancy within the cracks of the walls, while in localities with warmer winters continuing activity may take place. Only sporadic cases of infestation by *R. sanguineus* have been described in central and northern Europe.

Studies on infestations in some Mediterranean cities [35] showed that permanent populations of the tick are absent in apartments where dogs are present, even without any kind of ixodicide treatment. However, the tick is present and may occur in large numbers in small private gardens and kennels of houses (or even within houses) in the outskirts. These country-type houses are very common in the Mediterranean region. Hourly climate data recorded by probes installed indoor and outdoor in these different types of construction showed that adequate humidity is a critical factor for successful establishment of indoor populations [35]. In central Europe, there are no humidity restrictions for the development of the tick in the private gardens or kennels and spring and summer temperatures are the only limitation. Recent studies on climate features [36] have shown that particular events, such as the heat wave in Europe in 2003, can result in temporary conditions adequate for the development and molting of immature stages. It is clear that despite the endophilous nature of this tick species, climatic conditions in the outer environment are critical for its long-term establishment in an area. An increase of about 2-3°C in the average temperature from April–September could result in the establishment of permanent populations of the tick in regions of northern temperate Europe where it is currently absent.

3. Effects of Climate Change on the Seasonal Aactivity of Ticks

3.1. Winter Activity of Ixodes ricinus. It is common knowledge that the seasonal activity of *I. ricinus* nymphs and adults extends from March to October in most parts of central Europe, whereas the larval stage begins questing only in May, at least in Berlin forests (Kahl and Dautel, unpublished). In contrast to parts of the British Isles, any tick activity from mid-November to mid-February is unusual in that region. The strong influence that temperature can exert on tick activity patterns, even in the cold season, was demonstrated in eastern Germany in the extraordinarily mild winter of 2006/7. The continuously warm-to-mild autumn in 2006 (1st September to 30th November), which was 3.4°C warmer than the long-term mean for 1961–90, was followed by a winter (1st December to 28th February) 4.6°C milder than the long-term mean (http://www.dwd.de, data from Potsdam) with only two days with a maximum temperature <0°C. On prepared field plots in a Berlin forest, questing adult *I. ricinus* (Figure 2) were found on every observation date throughout the winter (early November to early March) and questing nymphal ticks were absent on only two out of 19 occasions [36]. Moreover, Dautel and colleagues collected 88 nymphs and seven adult *I. ricinus* in two man-hours by flagging 1000 m^2 in a nearby forest in mid-January 2007.

Figure 2: Dorsally marked adult female *Ixodes ricinus* questing on a wooden rod placed in a field plot for observation of *I. ricinus* questing activity in a Germany (Berlin) forest (Dautel et al. 2008 [37], with permission from *International Journal of Medical Microbiology*).

At the same locality on another occasion in mid-February, nymphal and adult questing tick abundance was still higher (temperature maximum on both days approximately 7°C).

This is a good example of how flexible the seasonal questing activity of this widespread vector tick can be if the temperature conditions change from the local norm. The unusual registration of four cases of human TBE (a notifiable disease in Germany) in early 2007 (early January to late February) shows that there was some tick questing activity in other parts of Germany as well during that winter. If winter temperatures generally increase in the future, it can be assumed that seasonal periods with no questing *I. ricinus* will become shorter in central Europe. It is evident that winter activity of vector ticks distinctly increases the risk for forest visitors of an infectious tick bite, especially if they do not expect ticks to be active at that time of the year. It is unclear, however, what the chances are for a winter-active tick to find a host at that time (because of reduced host abundance in winter though this may also change with the advent of warmer winters). It is also unclear how winter activity may affect the remaining seasonal activity pattern (winter-active ticks spend precious energy) or whether any changes in the seasonal activity of *I. ricinus* nymphs and adults are beneficial or detrimental for the perpetuation of tick-borne pathogens. The infection of *I. ricinus* larvae during feeding is a crucial step in the circulation of many tick-borne pathogens and the effects of higher ambient temperatures on the seasonal activity of larvae and its chances to find a suitable host may be of great significance in determining the prevalence of some tick-borne diseases.

3.2. Summer Activity of Ixodes ricinus. In 1976 the early summer weather in County Wicklow, Ireland, included maximum air temperatures of 29–31°C recorded on sheep pastures where *Ixodes ricinus* activity was being studied. Such monthly maxima in early summer may be the rule rather than the exception in this region in the coming decades [3]. Retrospective use was made of 1976 summer temperature and tick data in an examination of the effects of high temperatures on tick development and activity in relation to the predicted global warming [38]. In most parts of its range, *I. ricinus* shows some degree of bimodal seasonal activity and in 1975, more ticks were collected in autumn than in spring/summer, which may be attributed to the presence of hosts on these particular sheep pastures in late summer and autumn but not in spring or early summer for several years. However, by 1977, the pattern of tick activity had changed dramatically and more than 90% nymphal activity occurred from March to June. It was postulated that the elevated early and mid-summer temperatures of 1976 were the primary cause of the change from autumn to spring/summer-dominated nymphal activity.

This possibility was investigated by studies on tick development under quasi-natural conditions. The threshold period for deposited engorged larvae to enter a developmental diapause was identified as the first two weeks of August, after which time larvae overwintered in an engorged state and did not reappear as nymphs until the following autumn. The tick abundance data suggested that the 1975 autumn-feeding adults gave rise to larvae that fed predominantly in the prediapause period, so that they had the opportunity to overwinter as unfed nymphs and thus join the spring-active ticks in 1977. This interpretation was supported by a degree-day development model for *I. ricinus* originally described by Gardiner et al. [9], that predicted the appearance of larvae from autumn-laid eggs a month earlier when exposed to 1976 temperatures than when exposed to more normal temperatures [10]. The high summer temperatures of 1976 had apparently transferred ticks from an autumn-active cohort to a spring-active one. The process revealed by this study suggests that after hotter summers much of the host-seeking activity of *I. ricinus* will occur in late autumn, to a lesser extent in the winter months, and with strong activity again in early spring. Larval activity is likely to be mainly restricted to mid-summer (as long as humidity requirements are satisfied) with the majority of larvae avoiding developmental diapause and becoming active as nymphs in late autumn or early spring of the following year. Interestingly, this pattern of activity is very similar to that of the American *I. scapularis* in New Jersey, USA [39, 40], where air temperatures exceed 26°C for 50–60 days of the year. Suitable studies in southern Europe have not been undertaken, but a similar phenology to the predicted scenario for *I. ricinus* in hot summers was described in a recent comprehensive study in central Spain where maximum summer air temperatures generally reach 26°C [41]. It might be expected that with low precipitation in hot summers, survival and activity of ticks such as *I. ricinus,* which is very susceptible to desiccation, would be reduced. Indeed, it has been reported that in Switzerland saturation deficits depressed nymphal and adult *I. ricinus* activity [42]. However, Irish data [38] showed that all active stages of *I. ricinus* will quest throughout hot dry weather as long as appropriate vegetation cover is present to provide opportunities for rehydration. The same situation appears to obtain for *I. scapularis* immature stages in the USA [40] .

It seems likely that with increased global warming, *I. ricinus* activity will occur more in the autumn and winter months in many areas and, furthermore, a greater proportion of the tick population may be active at this time than at present, with a consequent temporal change in the risk of tick-borne diseases.

4. The Role of Modeling in Analysis of Climate Change Effects on Ticks

4.1. Climate Suitability Modeling. Climate suitability for a tick population can be defined as the fitness of a set of climatic conditions for the existence of that population in a given region. However, many other factors operating at different levels restrict the effective dispersal and establishment of potential invaders. Thus, while the climate in a particular location may be suitable for a given tick species, the potential for dispersal there and the ability to establish a new viable population may be very low. Furthermore, microclimatic variables such as soil surface temperature and relative humidity (which are affected by such things as slope and aspect, snow cover, vegetation, litter layer, humus and underlying soils) may be crucial in determining the distribution pattern of specific niches for tick survival within an area. Most data on climate preferences of ticks have been empirically derived from descriptions of the abiotic components of the environmental niche, as defined by the climate-supported native populations, and based on the assumption that they are homogeneously distributed in the native area.

The basic concept underlying species occurrence modeling is the definition of the ecological niche: each species is found within specific ranges for environmental variables that support individual survival and reproduction [43]. We refer here to climate instead of environmental or ecological space because these studies are aimed at understanding the relationship of ticks to climate, and ignore other basic aspects, such as vegetation patterns or host abundance, that are also involved in delineation of the "ecological" preferences of a tick species or population. Species occurrence can be predicted by inclusion of appropriate climate variables in what are commonly referred to as climate suitability models (CSMs): the relationships are generalized from a sample of correlations of species presence with specific values of environmental variables. While it is well recognized that the climate niche space occupied by a species across its geographic range may vary, this is rarely considered in current modeling approaches despite its obvious importance. When regional climate niche variations occur, a CSM derived for a particular area may not apply to other areas, and a model derived from a large area may have comparatively weak local predictive power. A widely held assumption in traditional models of tick distribution is that responses of species to environmental gradients are unimodal and symmetrical. Thus, climate suitability is predicted to decline from central (and ecologically optimal) areas of a species' range towards the periphery. In suboptimal conditions, a species may compensate for physiological stress by a shift in niche position. For wide-ranging species, suitable ecological conditions may vary considerably between different regions within the range.

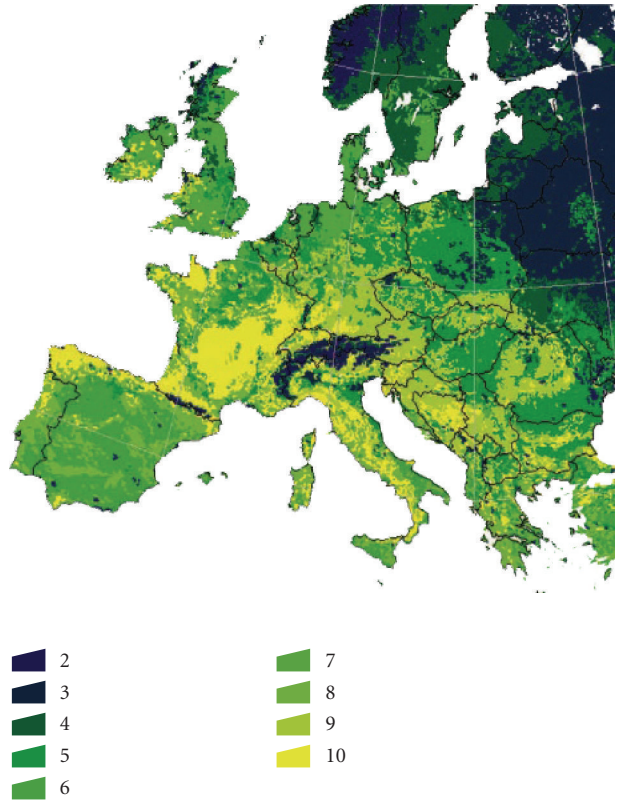


Figure 3: The vegetation-derived clusters as recognized over the western Palaearctic. The image was obtained from a yearly series of monthly satellite images, capturing the Normalized Difference Vegetation Index (NDVI): a measure of the photosynthetic activity of the vegetation. These images were subjected to a cluster analysis according to the monthly NDVI features to obtain 10 categories (category 1 is water and not displayed in the picture). (Estrada-Peña et al. 2006 [48], with permission from *Medical and Veterinary Entomology*).

However, these CSM are unsuitable if an adequate understanding of the factors operating over the transmission of a disease is necessary. The many variables involved in such processes, like hosts, densities of questing infected ticks, and a perception of the small scale of foci, are only adequately addressed with models designed to describe seasonal dynamics. While some models with biological content have been produced for tick species such as *Boophilus microplus* [44], *Amblyomma americanum* [45], and *Ixodes scapularis* [46], none are currently available for European ticks. Such models are a priority to adequately understand the impact of climate change on tick populations, provided that adequate data are used for important components such as host densities and microhabitat suitability. However, despite the absence of such data in climate suitability models, these models have proved useful in the elucidation of tick-borne disease foci for the recent outbreak of CCHF in Turkey [47].

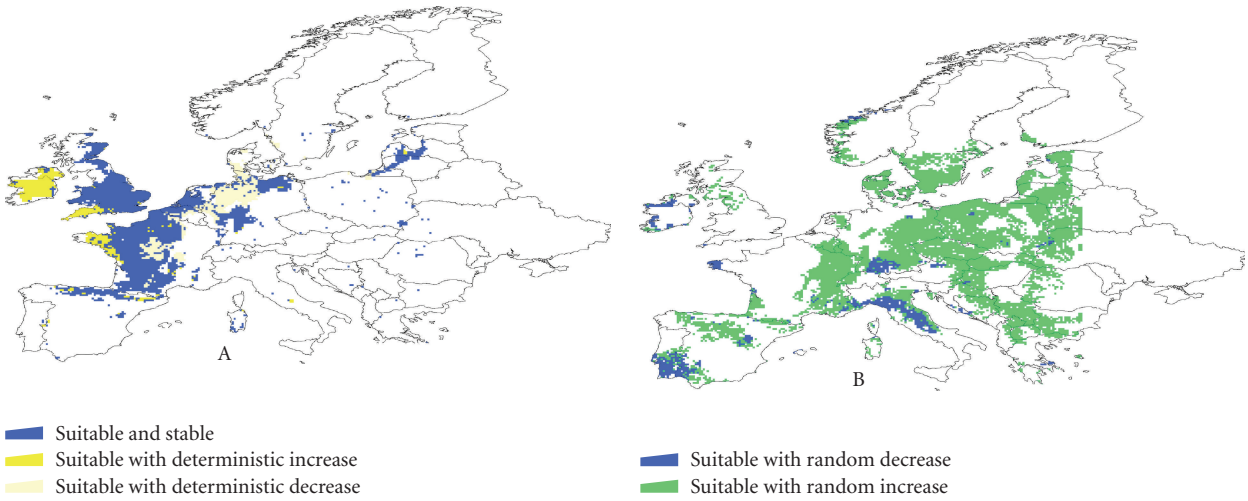


Figure 4: An analysis of the long-term changes in climate suitability for the tick *Ixodes ricinus* in Europe (1900–1999). A temporally extensively gridded dataset was subjected to a temporal analysis to understand how climate has changed in 100 years and how this trend affected the climate suitability for the tick. Areas are divided into suitable and unsuitable (the last, without colors in the figure). In the panel "A," the area marked as suitable and stable means no changes in suitability for the tick. Deterministic increase or decrease means a continued trend towards increasing or decreasing climate suitability. Panel B shows the areas where random walk trend has been observed. These areas are subjected to periodic cycles of climate, thus allowing cycles of increasing or decreasing climate suitability for the tick. (Estrada-Peña and Venzal 2006 [50], with permission from *Ecohealth*).

4.2. Recent (100 Years) Changes in Climate Suitability for Ixodes ricinus. Both regional climatic requirement variations and the existence of "demes" (defined as populations of closely related interbreeding organisms of the same species with differing responses to the wide array of climate factors occurring across the geographical range of the species) have been demonstrated for *I. ricinus* [47] (Figure 3).

The study revealed at least 10 distinct *I. ricinus* groups with a pattern of distribution closely overlapping with the presence of previously reported phenotypic forms of the species, and is in general agreement with a 16S mitochondrial rDNA study of genetic variation in *I. ricinus* [49]. The importance of these findings is that the climate and vegetation features correlated with the genetic groups in the whole tick metapopulation, with different populations having specific climatic requirements and unique genetic fingerprints. A study on the changes in climate suitability for *I. ricinus* in the western Palearctic has been carried out using deme-derived models based on the different populations recognized in the Palaearctic [50]. The distribution records available for the different demes were used to build partial models (i.e., applied to regions of the whole distribution range) from which a complete map for the whole region was produced. The study used a long (1900–1999) series of climate data at coarse resolution (10 minutes of arc) to examine the trends in climate and to estimate sustained variations in climate suitability for *I. ricinus*. While some areas showed a deterministic (i.e., continuous) tendency towards increasing or decreasing suitability for the tick, others showed unambiguous cycles of climate suitability, termed areas of random walk. In these, populations of the tick may undergo periodical variations in their geographical range as a consequence of cyclic changes in climate.

This analysis suggests that while climate suitability for *I. ricinus* did not change in a large area of Europe during the 100-year study period, it increased in specific geographically limited locations and decreased in others (Figure 4).

These changes are not recent and are associated with yearly and summer rainfall patterns rather than with temperature. The reported increased abundance of *I. ricinus* in parts of Europe (e.g., Sweden [11]) coincides geographically with the regions where a recent increase in climate suitability has been detected, within zones having a marked random walk tendency. Thus, the observation of higher tick abundance in recent years may not be due to a permanent shift in tick populations, but rather because the long-term climate cycle, which varies on a wide timescale, has been in a phase that is favorable to tick survival. No single variable was consistently associated in the study period with changes in climate suitability across sites where random walk was detected. The absence of a single regulatory variable seems to be connected with the different climate niche experienced by the tick populations in their distribution area. Thus, rainfall and temperature have different regulatory abilities according to the portion of the tick's climate envelope represented in a given area.

4.3. An Overview of the Climate Suitability for Ticks in the Mediterranean Region. The Mediterranean region is expected to experience profound changes in climate [51], and furthermore, its close proximity to the African continent makes it a particularly sensitive area to invasion by ticks currently restricted to northern Africa. It is, therefore, of interest to explore the predicted impact of climate changes on the suitability for ticks. The ticks involved (genera *Boophilus*, *Dermacentor*, *Rhipicephalus,* and *Hyalomma*) find

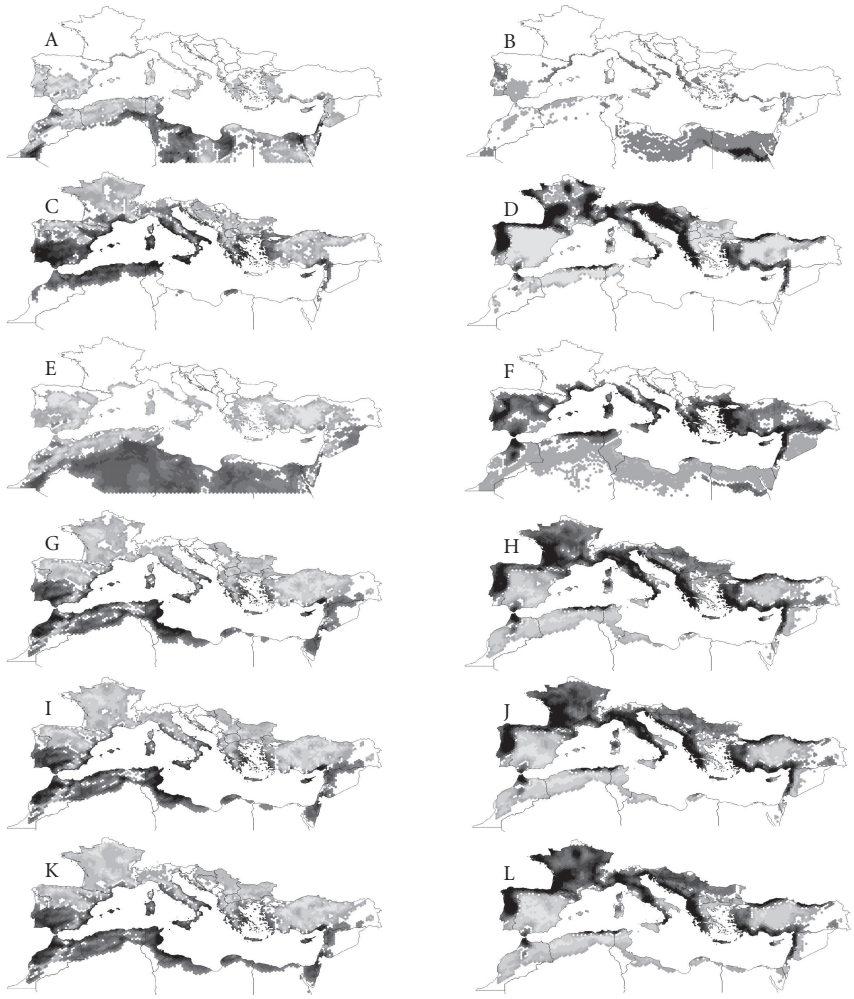


Figure 5: Predicted geographic impact (habitat suitability turnover) of different climate change scenarios. The maps shows the forecasted changes in habitat suitability for different tick species, with changes in temperature (left column) or rainfall (right column) analyzed by consensus analysis (a statistical method of classification using multiple input variables) to show the most coherent response to a range of changes in predictor variables. Dark shades of grey indicate increased climate suitability following a decrease in the predictor variable scenario (temperature or rainfall). Light shades of grey indicate increased climate suitability following an increase in the predictor variable. A and B: *B. annulatus*; C and D: *D. marginatus*; E and F: *H. excavatum*; G and H: *H. marginatum*; I and J: *R. bursa*; K and L: *R. turanicus*. (Estrada-Peña and Venzal 2007 [52], with permission from *Journal of Medical Entomology*).

their maximum suitability in Mediterranean type vegetation, including areas with cold winters and dry summers, and are prevalent in wide areas of that region, extending well into the Middle East. Most of them are vectors of pathogens of animals and humans. Predicted changes in climate are also expected to have a serious impact on the vegetation structure, driving the area toward a more open, brush-like vegetation, where these ticks find their ecological optima.

An estimation of the climate niches for each of these ticks has already been published [52]. Global climate models remain relatively coarse in terms of spatial resolution; this compromised the desired resolution of that analysis. To address this problem, new climate layers were created with monthly increases and decreases in temperature of 1 and 2°C, and monthly variations in rainfall of 60, 80, 120, and 140% of actual values. For each combination of temperature and rainfall, the climate suitability for each species in the region was evaluated. The baseline climate suitability was used as a framework to compute the impact of the different changes of climate over the expansion or retraction of the geographical range for each species. The expected impact is displayed in Figure 5.

For *B. annulatus* and *H. excavatum*, an increase in temperature would lead to an increase in suitability within regions of northern Africa and limited parts of southern Europe. Changes in rainfall are predicted to have little geographic impact on *B. annulatus* (which in any case is a one-host-tick that is much less exposed to ambient conditions than three-host-ticks), but they show a clear effect on *H. excavatum*.

In the case of *D. marginatus*, a decrease in temperature results in an increase in the extent of adequate suitability in

northern Africa, most of coastal southern Europe, and wide areas of southern Spain, whereas an increase in temperature would result in a northward expansion of the suitable habitat for this species. Changes in rainfall result in similar effects to those described for the above-mentioned changes in temperature. Large areas of Europe would potentially be affected by an increase in the climate suitability for *Rhipicephalus* spp. and *H. marginatum* after an increase in temperature and decrease in rainfall. Decreasing temperatures in Europe are predicted to result in habitat loss for *B. annulatus, R. bursa,* and *H. marginatum*. Again, we must stress that this is an evaluation of the climate suitability for these tick species, since changes in vegetation, host availability, and animal movements were not included in the models.

5. Effects on Tick-Borne Diseases in Europe

All the tick species considered in the earlier sections are important vectors of disease and an increasing incidence of these diseases is the most significant potential outcome of climate changes that affect ticks, directly or indirectly. As described above, there is little doubt that *Dermacentor reticulatus* is currently extending its range and this is reflected in the occurrence of canine babesiosis (caused by *Babesia canis canis*) in new areas (24, 25, 26, 27, 28). *Hyalomma marginatum* is one of the vectors of Crimean-Congo hemorrhagic fever (CCHF), which occurs in parts of Africa, Asia, the Middle East, and south-east Europe. The largest epidemic on-record occurred in Turkey, which started in 2002 and is still ongoing [47], and further north, a recent outbreak was reported in the Balkans, another known endemic area [53], but there is no evidence as yet that this disease is spreading further northwards. However, as discussed above, the potential for the introduction and establishment of vector populations in areas of predicted climate suitability is increasing.

Rhipicephalus sanguineus is the primary vector of Mediterranean-spotted fever (a rickettsial zoonosis caused by *Rickettsia conorii*), and also *Ehrlichia canis* and *Babesia canis vogeli* (causing, resp., rickettsial and protozoal diseases of dogs). Although *R. sanguineus* can establish in kennels in central and northern European latitudes, thus potentially causing short-lived localized disease outbreaks, sufficient survival in the external environment does not seem to occur for significant disease transmission in northern temperate Europe. Nevertheless, concerns about its possible introduction and establishment have prompted the authorities in some countries, such as the United Kingdom and Ireland, to make treatment against ticks a component of the pet-passport scheme.

Ixodes ricinus is the most abundant and widespread tick in Europe and together with the Eurasian species, *I. persulcatus*, transmits Lyme borreliosis (LB) and tick-borne encephalitis (TBE). LB occurs at a relatively high incidence for a zoonotic disease, ranging from 155 per 100,000 in Slovenia to 0.6 per 100,000 in Ireland [54]. However, it is difficult to statistically relate LB incidence to climate change because the reliability of incidence data is uncertain due to diagnostic problems and limited or absent reporting in most countries. Nevertheless, in some regions, it has proved possible to relate disease incidence to climate, and a positive association of incidence with mild winters and warm, humid summers was reported in southern Sweden [55]. The suggested effect of mild winters is possibly due to an extension of the tick activity season, resulting in increased numbers of infected ticks becoming available in the following year. Warm humid summers might result in a more efficient transmission by the vector, but there is limited evidence for this and the authors suggest that the observed increased LB incidence may be due to increased human exposure in these conditions.

Since TBE is notifiable in most countries where it occurs, current incidence data are more reliable than those for LB, even though reporting standards differ between countries and there are increasing differences in TBE vaccination rates between countries. Increases in Swedish cases since the mid-80s were associated with two consecutive years with milder winters, earlier arrival of spring and prolonged autumn periods with temperatures above 5–8°C [13]. The possibility that this is caused by climate effects on ticks is suggested by the northward extension of *I. ricinus* distribution [11, 12]. Similarly, an upward movement of the TBE prevalence altitude ceiling correlating with increasing temperatures has been reported [15, 56], which accords with reports of increasing numbers of active ticks at higher altitudes [15].

Detecting climatic effects on the incidence of LB and TBE in areas close to the latitude and altitude distribution limits of the vector is less complex than in other parts of Europe where the direct and indirect impacts of climate change on disease incidence are often confounded by other factors. Such studies should either be based on reliable long-term historical datasets in an area (e.g., the 40 year surveillance program for TBE in Stockholm County, Sweden [13]) or based on data from different regions or countries, collected with similar methodologies (e.g., vector sampling and analysis) and subject to similar variations (e.g., TBE vaccination rates and reporting criteria). There is a need for a pan-European surveillance network, for example based on the abundance of infected vectors, as suggested by WHO [57].

Randolph et al. [58] correlated the occurrence of synchronous activity of *I. ricinus* larvae and nymphs (resulting in cofeeding transmission) with that of TBE cases, and also reported a relationship between these two variables and the rate of decline of autumn temperatures. It, therefore, seems likely that climate change will affect the dynamics of TBE transmission, thus altering the distribution of the disease [59], but the exact mechanisms of this interaction remain to be elucidated.

Despite the ready availability of data on TBE incidence, a firm causal relationship with climate change remains elusive. A detailed study of the records of TBE incidence over the last 2-3 decades in Estonia, Latvia, and Lithuania showed that although TBE incidence rose dramatically in some areas, there was so much heterogeneity in the data that it was impossible to identify climate change as the main factor driving increased disease incidence. It was concluded that the many socioeconomic changes arising from the end of

Soviet rule probably acted synergistically with climate factors to increase TBE incidence [60].

6. Conclusions

Transmission of infection occurs when thereis an overlap of activities between reservoir, vector, and humans, and differs according to the pathogens and the location. Climate change may impact all of these stages and their interactions. Although changes in climate and in the length of the different seasons will directly affect tick survival, activity, and development, there is no good evidence that rising temperatures will result in a greater abundance of ticks by simply increasing rates of development; rather changes in development rates will make tick cohorts available to different diapause windows (largely determined by day length), thus changing patterns of seasonal activity [38]. Indirect effects of climate change will impact the number of infected ticks by affecting vegetation. For example, a warming climate in central Europe is likely to result in a decrease of Norway spruce (*Picea abies*) and the areas involved will probably be colonized by beech (*Fagus sylvatica*) [61], the fallen leaves of which provide a favorable microclimate for survival of the free-living tick stages. Additionally, climate change will also have indirect effects on tick-borne pathogen transmission by affecting the survival and abundance of tick maintenance hosts, such as deer, and pathogen-reservoir hosts such as rodents and birds. Climate change may also influence disease risk by affecting the long-term use of land (e.g., farming, tourism, etc.), and weather patterns have an effect by influencing short-term human behavior such as picnics and mushroom picking. Climate effects are more easily noticeable close to the geographical distribution limits of both vector and disease. The magnitude of the effects of climate change in an endemic area depends on local conditions and vulnerability, and is determined not only by ecological conditions but may be influenced by socioeconomic factors, human migration and settlement, ecosystems and biodiversity, migrating patterns of birds, land-use and land cover changes, human cultural and behavioral patterns, and immunity in the population. Since some of these conditions are in turn influenced by climate change, a complex chain of processes exists that makes the precise factors responsible for changes in disease incidence often difficult to determine [54]. A further difficulty in determining future scenarios is presented by the fact that the predominant tick species in Europe, *Ixodes ricinus* is extremely flexible and adaptable and can exhibit rather different seasonal activity even in adjacent parts of its geographical range. Much current research effort attempts to match datasets collected for different purposes and in order to reduce confounding variables, it is evident that data from long-term studies on disease incidence, tick biology, tick distribution and tick abundance, host abundance and distribution, and relevant vegetation biology, specifically in relation to climate change, are required [62]. Such data will permit the development of models to predict future tick-borne disease scenarios, which take account of dynamic biological processes instead of simply the likelihood of occurrence of climate suitability for particular tick species.

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Review Article

Cryptococcus gattii: Emergence in Western North America: Exploitation of a Novel Ecological Niche

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The relatively uncommon fungal pathogen *Cryptococcus gattii* recently emerged as a significant cause of cryptococcal disease in human and animals in the Pacific Northwest of North America. Although genetic studies indicated its possible presence in the Pacific Northwest for more than 30 years, *C. gattii* as an etiological agent was largely unknown in this region prior to 1999. The recent emergence may have been encouraged by changing conditions of climate or land use and/or host susceptibility, and predictive ecological niche modeling indicates a potentially wider spread. *C. gattii* can survive wide climatic variations and colonize the environment in tropical, subtropical, temperate, and dry climates. Long-term climate changes, such as the significantly elevated global temperature in the last 100 years, influence patterns of disease among plants and animals and create niche microclimates habitable by emerging pathogens. *C. gattii* may have exploited such a hitherto unrecognized but clement environment in the Pacific Northwest to provide a wider exposure and risk of infection to human and animal populations.

1. Introduction: The Organism and the Disease

Cryptococcus gattii (formerly, *Cryptococcus neoformans* var. *gattii*) [1] is a basidiomycetous yeast pathogenic to immunocompetent mammals including humans. This relatively uncommon organism differs from the congeneric, more commonly encountered pathogen, *C. neoformans*, with regards to phenotypic characteristics, natural habitat, epidemiology, ecology, clinical manifestations of disease, and responses to antifungal therapy [2]. Phylogenetic studies have shown that *C. gattii* and *C. neoformans* diverged from a common ancestor approximately 40 million years ago [3]. Hosts acquire cryptococci via inhalation, and the disease ("Cryptococcosis") caused by both *C. gattii* and *C. neoformans* affects the lungs, with the potential to disseminate to distant tissues, most frequently, the central nervous system, causing life-threatening meningoencephalitis [4]. Compared to *C. neoformans*, *C. gattii* infections more often cause granulomatous lesions (cryptococcomas) in lung and brain, with more associated neurological sequelae and morbidity [5–8]. Though otherwise healthy hosts presenting with meningitis respond to antifungal therapy, complete mycological cure (culture negativity) appears to be more often delayed [7, 9]. Genetic typing studies using molecular techniques have identified distinct haploid genotypes among the clinical, veterinary, and environmental isolates of *C. gattii*, namely, VGI, VGII (further subdivided into VGIIa, VGIIb [10, 11], and recently VGIIc [12]), VGIII, and VGIV [11–15]. The genotype VGIIa was preponderantly present in the emergence of *C. gattii* disease in 1999 on Vancouver Island and Lower Mainland of British Columbia (BC) in Canada, and has, therefore, been termed the "Vancouver Island major emergence strain"; it was found to be unique to the Pacific Northwest and hypervirulent in mouse studies [10, 11].

2. *C. gattii* Outbreak in the Pacific Northwest

C. gattii emerged as an agent of life-threatening infections in the Pacific Northwest of North America in 1999. Previously unknown in the region, more than 200 cases of *C. gattii* have now been documented in humans, and more than twice that number in domestic animals, accounting for an average annual incidence of 6.5 cases per million in BC or 27.9

cases per million on Vancouver Island; this is the highest "endemic" incidence reported worldwide [16]. There has been a substantial mortality from this disease; between 1999 and 2006, the case fatality rate from *C. gattii* disease was estimated to be 4.5% [17].

Although the majority of locally acquired *C. gattii* cases occurred in Vancouver Island residents, since 2004 there has been a steady increase in the numbers among BC Lower Mainland residents [17] as well as among the Northwestern states in the United States (Washington and Oregon) [10, 11, 16, 18–20]. To date, we are aware of approximately 20 cases of *C. gattii* disease diagnosed in humans in Washington and Oregon, many of whom have had no travel history to any known *C. gattii*-disease endemic zone (K Marr, unpublished observation). The organism has also been recovered from the environment in Washington State [16]. An overwhelming majority of these isolates belonged to the molecular genotype VGII (~95% VGIIa; ~5% VGIIb) and one VGIII [10–12, 14, 16, 19, 20]. Though the exact mode of transport of *C. gattii* from Vancouver Island to the Lower Mainland is not known, studies of possible dispersal mechanisms have indicated association of *C. gattii* cases with high-traffic locations, and evidence of human-associated dispersal through wheel wells of cars, shoes, and movement of soil or wood products as well as water [18, 21].

Since 1999, the incidence of *C. gattii* infection in domestic animals has increased greatly in Western North America, in parallel with the outbreak of human cryptococcosis. *C. gattii* disease has been diagnosed in dogs, cats, ferrets, porpoises, alpacas and llamas, horses, and psittacine birds on Vancouver Island, BC Lower Mainland [22–24] as well as in Washington and Oregon [12, 16, 20]. Cryptococcal infection in animals occurs from inhalation of airborne infectious propagules (yeast or spores) and subsequent colonization of the nasal cavity and paranasal sinuses, often resulting in asymptomatic carriage and/or subclinical infection [2, 22]. Subclinically infected animals may clear the organism, remain protractedly colonized and/or infected (with repeatedly positive serum for cryptococcal antigen), or progress to clinical disease [25]. Disease can be manifested by upper respiratory symptoms, subcutaneous nodules, pneumonia, central nervous system and/or ocular disorders, and lymphadenopathy [2, 24]. A unique feature of the Pacific Northwest outbreak is the number of marine mammals, primarily porpoises, which have died with *C. gattii* pneumonia [24]. Over 90% of the veterinary isolates have belonged to molecular type VGIIa [16, 22–24]. Identified risk factors in animals in this region include disturbance of soil and/or human activities such as logging within 10 km, hiking, or hunting in areas colonized with *C. gattii* [26].

3. *C. gattii:* Establishment in a Novel Environmental Niche

In a seminal study on the epidemiology ofcryptococcosis [27], it was noted that the majority of clinical cases of *C. gattii* disease occurred in people residing in hot and humid climates of the tropics and subtropics. Prior to 1999, the geographical regions with any degree of *C. gattii* endemicity included Australia and New Zealand, Papua New Guinea, South and Southeast Asia (Cambodia, Malaysia, Thailand, Vietnam, China, Taiwan, Singapore, Nepal, and the Indian subcontinent), parts of Latin America (Argentina, Brazil, Colombia, Uruguay, Paraguay, Peru, and Venezuela), Southern California, Mexico, Hawaii, Central and South Africa, and certain parts of Europe (Austria, Germany, France, Italy, Greece, and Spain) (reviewed in [2]; also see [28–32]).

Studies from Australia and elsewhere [2] indicate that *C. gattii* can colonize the environment in tropical, subtropical, temperate, and dry climates. Although *C. gattii* grows slower at 37°C compared to *C. neoformans* isolates [33], environmental isolates of *C. gattii* grow equally well at 30°C and 37°C—a survival property believed to be mediated in part by a manganese-containing mitochondrial superoxide dismutase (*SOD2*) which is induced at elevated temperatures [34]. Recent work with environmental isolates from Vancouver Island has shown that the organism, when grown in nutritionally deficient soil extract broth, uses constituents in the soil to produce melanin; these melanized cells are more resistant to UV irradiation exposure than the same strains which did not melanize when grown in 1/16th strength malt extract. The ability of this *C. gattii* genotype to resist UV irradiation and colonize with high concentrations in desiccated soil may contribute to the survival and dispersal of this strain in this environment [35].

The origins of the *C. gattii* strains, which now permanently colonize the environment on Vancouver Island, remain unknown. The provincial medical mycology collection has been examined, and no archived *C. gattii* isolates were identified prior to 1999. Significant global dispersal of cryptococcal strains [14, 36] makes accurate determination of specific origin difficult. However, genotypic analysis enabled the study of lineages of the *C. gattii* isolates, tracing the origin and evolution of the organism and establishing its relevance in the context of the outbreak. All *C. gattii* clinical isolates from the Vancouver Island emergence were found to contain α allele of the mating type-specific genes (MATα) and were sexually fertile [11, 37]. Genotypic analysis further revealed that a rare, nonclassical, same-sex reproduction between two MATα parents resulted in the VGIIa strain. These observations support the hypothesis that cryptic same-sex reproduction may have enhanced the virulence of the VGIIa genotype, helping a "hypervirulent" strain adapt to and propagate in the local environmental niche [10]. In contrast, study of Australian *C. gattii* populations recovered from Eucalyptus tree hollows found that the organism exists as either α-mating-type isolates, or both a and α-mating-type isolates, and both unisexual and heterosexual recombination produce infectious spores [38].

Because of global dispersal [14, 36], it is possible that the Vancouver Island major genotype VGIIa may exist elsewhere, but that remains to be established; one South American isolate thus far reported to be highly similar to VGIIa differs at one locus by multilocus sequence typing (MLST). It may well be related to the major outbreak strain, but either with a different origin (in Australia, South America, or within the

Pacific Northwest), or by genetic drift (in transit to the Pacific Northwest, perhaps by mating with another VGII strain). On the other hand, the minor genotype VGIIb appears to be identical with fertile isolates from Australia and likely originated there [10].

In the first two Oregon cases of nontravel-associated *C. gattii* disease, *C. gattii* isolates genotyped as VGII, but they were genetically distinct from either VGIIa or VGIIb strains found on Vancouver Island [16] and the relationship of these cases to the outbreak is unknown. However, two archived *C. gattii* isolates from the United States, NIH444 (a.k.a. ATCC 32609 and CBS6956) and NIH B4534 (a.k.a. CBS7750), were found to be identical to the Vancouver Island VGIIa strain [10, 11], leading to the hypothesis that this genotype may have been present in North America for more than 30 years. Strain NIH444 was isolated from a sputum sample from a patient in Seattle, Washington, in the early 1970s, and strain NIH B4534 was recovered from a Eucalyptus tree in San Francisco in 1992. It is important to note that we do not have a complete travel history from the Seattle patient. However, if this isolate has been in the environment in Western North America for this long, other factors, such as changing conditions of climate or land use and/or host susceptibility, have appeared to encourage its emergence in more recent years.

4. Climate Change and Disease Relationship: Overview

In the last 100 years (1906–2005), the global temperature has increased by 0.74 [CI, 0.56–0.92]°C; and the linear warming trend over the last 50 years (0.13 [CI, 0.10–0.16]°C per decade) is nearly twice that for the last 100 years [39]. The last three decades have seen an unprecedented escalation of global warming—both at the level of the middle troposphere and the surface—a large part of it being anthropogenic through emission of greenhouse gases. Long-term changes in climate and subsequent effects have been observed at continental, regional, and ocean basin scales, including changes in Arctic temperatures and ice, widespread changes in humidity and precipitation amounts, ocean level and salinity, wind patterns, and aspects of extreme weather including droughts, heavy precipitation, heat waves, and the intensity of tropical cyclones. It has been predicted that the earth in the 21st century will face some 2°C (3.5°F) or more in additional warming [39]. However, the rate of change between microenvironments varies substantially, with polar zones changing more quickly while other zones remain relatively stable, and therefore, global averages cannot be used to predict effects of microclimates. Since 1948, the average annual temperatures at one site on the eastern coast of Vancouver Island increased by 1.44°C which is a statistically significant change ($P < .001$) (data obtained from Environment Canada). Unfortunately, many government sponsored meteorological stations have been abandoned, leaving research scientists with the arduous task of documenting microclimate variation.

Speculative studies on how a widespread climate change, specifically the elevation of global temperature, might affect the distribution of infectious diseases began about two decades ago. Health outcomes of climate change are diverse, and depend upon many different factors with respect to biodiversity, nutrient cycle, physical relocation, internal defense systems, and transmission dynamics within microbes [40]. The initial studies almost exclusively focused on vector-borne diseases, predicting possible vector movements with rise in temperature in erstwhile temperate zones. However, the relationship between climate change and infectious disease is inherently complex, and not easily amenable to predictive epidemiology. For example, the impact of climate change on infectious disease is not restricted to vector-borne diseases or infections that affect human health directly. Climate change may influence patterns of disease among plants and animals, impacting the human food supply (and thereby reducing human resistance to infections), or indirectly causing human disease patterns to shift, as the host range for disease reservoirs may change because of human migration to geographically disparate areas and/or changes in abundance and distribution of disease vectors and agents [40–42].

In 2004, the World Health Organization (WHO) published a study of the global disease burden attributable to human-induced climate change up to the year 2000, and made quantitative model-based predictions of climate change-associated health risks up to 2030 [43]. Despite the lack of comprehensive models for various specific climate-disease relationships, overall results indicate that even the subtle climatic changes occurring since the mid-1970s could be responsible for over 150 000 deaths from climate-sensitive diseases, and approximately 5 million disability-adjusted life years each year—the most vulnerable being the poorer regions of the world, according to WHO estimates, although climate change poses a global threat to public health [43]. Diseases with which significant association of climate changes has already been observed include water- and food-borne diseases (such as cryptosporidial diarrhea, salmonellosis, algal toxicity, and cholera), vector-borne diseases (such as malaria, Chagas disease, borreliosis, schistosomiasis, and dengue), rodent-borne diseases (such as leptospirosis) as well as infections caused by the St. Louis Encephalitis virus and the West Nile virus, both of which have been shown to prefer warmer climates [42]. The global warming phenomenon has, in addition, been associated with various other noninfectious diseases with significant human morbidity, including chronic respiratory ailments, cardiovascular diseases, neurological and psychiatric disorders as well as those pertaining to occupational health [44]. According to the WHO estimates of morbidity and mortality associated with anthropogenic climate change, the year 2030 would see a doubling of excess risk of the various health outcomes [43].

5. *C. gattii:* Change of Environmental Niche or Adaptation to a New One

In Australia, in the early 1990s, Ellis and Pfeiffer observed a correlation between the distributions of human cryptococcal disease and of Eucalyptus trees [45, 46], whose bark is rich in

dihydroxyphenylalanine (L-DOPA), a substance metabolized by *Cryptococcus* species to produce melanin; this has been hypothesized to contribute to their environmental survival [47, 48]. Association of *C. gattii* with Eucalyptus has been sporadically reported from India, Brazil, Italy, and the United States [49–52]. However, the absence of this association with Eucalyptus in other endemic areas, such as the Northern Territory of Australia [53], Papua New Guinea [54], Central and South Africa [55], Brazil, and Malaysia as well as the isolation of *C. gattii* from non-Eucalyptus trees and tree materials [56–60], indicates the existence of additional environmental sources. Since it was first discovered to have a stable ecological niche on Vancouver Island in 2002, *C. gattii* has consistently been recovered in high concentration from native trees, soil, air, freshwater, and seawater [16, 19].

Does the Vancouver Island outbreak, then, point to a changing ecological niche for this organism in Western North America? From the beginning of the *C. gattii* epidemic on Vancouver Island and adjoining areas, epidemiological studies showed that all humans and animals with cryptococcal infection either lived within or traveled to areas identified by unique weather and vegetation zones. The British Columbia Ministry of Forests categorizes areas with similar ecologies within the province by biogeoclimatic zone designations. A biogeoclimatic zone is a geographical area with an ecosystem comprised of a relatively uniform macroclimate, defined vegetation, soils, and animal life inhabiting that climate, and may contain smaller ecosystems (subzones) that reflect differences in regional climate, soil moisture, soil nutrient status, and environmental disturbance [61]. The unique zones along the eastern edge of Vancouver Island are in the rain shadow of the Olympic Mountains located in Washington State to the south, and include the Coastal Douglas Fir (CDF) and very dry Coastal Western Hemlock (CWH) biogeoclimatic zones. The CDF and very dry CWH zones are characterized by warm, dry summers (average summer temperature $15.6 \pm 1.24^{\circ}$C; 190.24 ± 55.5 mm rain), and mild, wet winters (average winter temperature $5.77 \pm 0.64^{\circ}$C; 884.33 ± 206.22 mm rain). *C. gattii* has been repeatedly and consistently isolated from the environment in these biogeoclimatic zones [16, 19, 22]. The CDF and CWH zones also include the Southern Gulf Islands and portions of the BC Lower Mainland. Similar climates with comparable temperature and rainfall extend further south into Washington and Oregon in the United States. The San Juan Islands, Puget Sound in Washington, and the Willamette Valley in Oregon harbor ecologically similar plant diversity as in BC, lending support to the idea that environmental niches suitable for colonization by *C. gattii,* are present in Western North America [16]. Indeed, large-scale environmental sampling performed during 2001–2005 on the BC mainland, the Gulf Islands, and Washington revealed that 3% of 2 033 off-Vancouver Island samples of air, water, soil, and trees were positive for *C. gattii* serotype B (mostly VGIIa, except two VGIs) [16].

Environmental sampling studies on Vancouver Island revealed high concentrations of *C. gattii* in the soil, indicating a potential source of exposure [18], and data gathered from the BC environment conclusively demonstrate that *C. gattii* is well adapted for survival in a dry, nutrient-deprived soil and is more likely to spread as airborne propagules during dry summer weather [19]. However, whereas *C. gattii* was consistently isolated from localized areas, it was not found, or was below the limit of detection in other areas, such as the San Juan Islands [62], despite ecological similarity to identified zones of endemicity in Vancouver Island and BC Lower Mainland. This suggests that there may be environmental "hotspots", *that is,* zones of high concentration, of *C. gattii* within the same broad ecological niches. There are also areas with transient colonization, with sites intermittently positive over time, and some sites which initially tested negative which subsequently either became colonized, or the concentration rose above the limit of detection [18, 19]. Whether microclimatic conditions govern the creation and maintenance of these hotspots is not known, but the fact remains that *C. gattii* now inhabits a stable ecologic niche in the environment of the Pacific Northwest in concentrations high enough to pose a risk of infection through environmental exposures. Prior to the outbreak of *C. gattii* disease, there was no reason to seek its presence in the environment, as there were no cases of locally acquired infection, and *C. gattii* is not a phytopathogen. The relationships between an emerging pathogen and the environment are not often studied, and it is even rarer that researchers have been able to document the emergence with as much detail as has been possible in this BC outbreak. The unexpectedly rich dataset obtained from BC environmental and epidemiological ecologies can be used to inform future eco-health investigations. In this case, the existence of libraries of cryptococcal strains from global clinical and environmental origins provided the resources for swift characterization and molecular epidemiology of the causative organism; this information changed forever the simplistic idea of unvarying geographic boundaries on infectious organisms.

A promising tool which can be used by climatologists working with epidemiologists to predict public health impacts is ecological niche modeling [63–65]. These computer simulations take into account ecosystems, meteorology, and the presence of pathogens in the environment to predict areas with similar ecologies that may be at risk of pathogen spread. A recently completed thesis from the University of British Columbia using the Generic Algorithm for Rule-set Prediction (GARP) modeled the potential for *C. gattii* to spread to other areas of the Pacific Northwest [66]. Using existing data from human and animal clinical cases and Geographic Information System (GIS) coordinates of colonized environmental sites [18, 19, 21, 24], the model predicts a larger area of potential colonization than is currently the case. The predicted area includes cities with large populations (over 4 million). Since this model was developed, there have been at least four-human, eight-animal, and four-environmental samples of *C. gattii* recovered from the predicted zone which was not on Vancouver Island [16, 17, 20]. By the same token, the vastly larger land mass of British Columbia is predicted not to become colonized with *C. gattii* in the foreseeable future due to the

extremes of temperature, snow cover, or lack of suitable habitat [66].

C. gattii, similar to the congeneric pathogen *C. neoformans*, has evolved mechanisms which protect it from the environment. For example, the melanin production by these organisms contributes to the protection from the ultraviolet radiation in sunlight. The predominant genotype of the Vancouver Island outbreak thrives in dry, nutrient poor soil at high concentrations. It appears to be well adapted for this marginal microecologic zone [19]. In Colombia, for example, the dispersal of *C. gattii* is greater during periods of high humidity or rain [67]. In Australia, the airborne distribution of propagules is associated with the flowering season of Eucalyptus [68]. Neither of these conditions describes the experience of the British Colombian genotypes VGIIa or VGIIb, which suggests that the organism is successful in this new ecologic niche because it can adapt preexisting characteristics to fill novel, underutilized microscopic niches, rather than strictly requiring a constrained environment. Even the widely held view that *C. gattii* was restricted to a symbiotic relationship with Eucalyptus trees has since been shown not to be the case, as *C. gattii* regularly colonizes native trees in countries outside Australia [57, 58, 69].

Cyclical climate change patterns (called "oscillations") are driven by forces such as solar cycles and the ocean currents characterized by the El Niño and La Niña years, whereas more long-term changes, such as an elevation of global temperatures, must be measured over decades or centuries. Therefore, the problem of association of climate changes with the epidemiology of various infectious diseases is twofold. Climate changes may be long term, with the potential to cause significant epidemiological changes over long-time horizons, or the climate may suddenly shift, changing patterns and spread of exposure in a given climatic zone. Both of these possibilities pose challenges to public health studies.

That *C. gattii* emerged as a human and animal pathogen in the late 1990s, in a new habitat, is indisputable. Whether or not the climatic conditions (both short- and long-term) in the new habitat made it conducive for the organism to do so is under debate. The oscillatory climate change patterns preceding the emergence were similar to the patterns seen in this area over the last 30 years. The years 1992–1994 and 1998–2003 were dryer than the 30-year average during the summer, followed by the years 1997, 2004, and 2005, which had higher-than-average summer rainfall; the winter rains roughly followed the same pattern. Importantly, however, the amount of snow coverage on southern Vancouver Island and the CDF biogeoclimatic zone decreased over the same time period. This constantly repeating dry/wet pattern in conjunction with the elevation of temperature may well be a driving factor in the prolongation of *C. gattii* as a pathogen in this geographic location. However, given the paucity of long-term data on *C. gattii* emergence in relation to climate changes, it is difficult to attribute the emergence entirely to climate change. A proof-of-concept example supporting the notion is *Coccidioides immitis*, an environmental organism which proliferates in wet years and is widely dispersed in dry years, causing localized spikes in hospitalization for areas adjacent to endemic locations [70]. For *C. gattii*, some factor(s) (climate, land use, human agency, etc.) must have become permissive to the permanent colonization of the organism in high enough concentration to cause disease with an incidence rate of around 28 cases/million population (on Vancouver Island alone) [16]. Identification of those factors would help formulate preventive public health approaches.

It is also clear that *C. gattii* disease has escalated to an "off island" problem as well, involving the Northwestern US (Washington and Oregon). The extent of disease and the distribution of the cryptococcal isolates within the United States are unknown, and will remain underappreciated until laboratory methods to identify and speciate the isolates, standardized reporting of disease, and environmental studies are established.

C. gattii already had the ability to survive in a wide range of environmental variations, but the Western North America outbreak teaches us that it may exploit hitherto unrecognized but clement environments and provide a wider exposure, and thereby, risk of infection to the human and animal populations. The challenge for public health is to coordinate efforts toward early recognition of the emergence of new or reemergence of previously encountered infectious diseases to alert primary health care providers as to the diagnosis and appropriate treatment in order to prevent excess morbidity and mortality. However, understanding the consequences of ecological change is a group effort which includes health researchers, climatologists, and ultimately global citizens whose health may depend on the capacity to adapt to a rapidly changing environment.

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Review Article

Paleopathology of Human Tuberculosis and the Potential Role of Climate

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Both origin and evolution of tuberculosis and its pathogens (*Mycobacterium tuberculosis* complex) are not fully understood. The paleopathological investigation of human remains offers a unique insight into the molecular evolution and spread including correlative data of the environment. The molecular analysis of material from Egypt (3000–500 BC), Sudan (200–600 AD), Hungary (600–1700 AD), Latvia (1200–1600 AD), and South Germany (1400–1800 AD) urprisingly revealed constantly high frequencies of tuberculosis in all different time periods excluding significant environmental influence on tuberculosis spread. The typing of various mycobacteria strains provides evidence for ancestral *M. tuberculosis* strains in Pre- to early Egyptian dynastic material (3500–2650 BC), while typical *M. africanum* signatures were detected in a Middle Kingdom tomb (2050–1650 BC). Samples from the New Kingdom to Late Period (1500–500 BC) indicated modern *M. tuberculosis* strains. No evidence was seen for *M. bovis* in Egyptian material while *M. bovis* signatures were first identified in Siberian biomaterial dating 2000 years before present. These results contraindicates the theory that *M. tuberculosis* evolved from *M. bovis* during early domestication in the region of the "Fertile Crescent," but supports the scenario that *M. tuberculosis* probably derived from an ancestral progenitor strain. The environmental influence of this evolutionary scenario deserves continuing intense evaluation.

1. Introduction

Today, tuberculosis is still a major predator of mankind causing every year millions of deaths worldwide. Despite the enormous social and economic burdens, the origin as well as the evolution of the pathogen is still far from being completely understood. This includes the understanding of the mechanisms of virulence, the host-pathogen interaction, and the bacterial development, including the influence of changing environmental conditions, including climate.

Tuberculosis as a chronic infectious disease is caused by mycobacteria. These mycobacteria are able to induce a chronic destructive inflammation that typically contains granulomas with central necrosis (the "tubercle"). Due to local and/or systemic spread principally every organ can be affected. Frequently, any systemic spread by the blood stream involves bone tissue with the most preferred osseous lesions occurring in the vertebral bodies.

Out of hundreds of mycobacterial strains—that mostly exist as soil bacteria—only four strains are the main infectious agents for human tuberculosis. All four strains, termed *M. tuberculosis, M. bovis, M. africanum, M. canetti*, lead to an identical clinical pattern. Few other mycobacteria, such as *M. avium* and *M. kansasii*, may also induce granulomatous pulmonary infection, frequently, but not exclusively in immunocompromised patients, such as in HIV-infected individuals. These mycobacteria are counted into the atypical mycobacterioses.

Until recently, the aspects of origin and evolution of pathogens were restricted to the comparative analysis of present isolates and the extrapolation of the resulting observations to evolution and origin. Despite considerable efforts, the resulting data remain hypothetical. The most recent technical improvements of polychain reaction (PCR) techniques, however, now allow the identification and characterization of gene segments in biomedical remains of hundreds to

thousands of years of age [1]. This has been made possible since PCR can amplify even minute amounts of DNA, such as the very small amounts of intact DNA-molecules residing in ancient biomaterial, for example, in bone or mummified soft tissues. This novel approach offers not only potential insight into the evolutionary time course of distinct strains of mycobacteria, but possibly provides also correlative data on environmental influences on mycobacterial development and its diversity.

In this report we describe the current status of ancient mycobacterial DNA-analysis in ancient human remains with particular reference to the present scenario of the evolution of human tuberculosis and propose initial assumptions on the influence of environment factors.

2. Previous Theories of the Evolution of Human Pathogenic Mycobacteria

Human tuberculosis has previously been assumed to have come from the close contact between humans and animals, especially from either bovine or caprine source. Accordingly, the bovine form of mycobacteria (*M. bovis*) had previously been suggested to be the initial evolutionary form of mycobacteria [2]. In consequence, the climatic changes in the Near East/North Africa with desert formation in distinct areas and the formation of the so-called "Fertile Crescent" lead to the gathering of nomadic populations and the formation of sedentary settlements. Along with this change in human live style, animal domestication took place. This was regarded to be an initializing event of TB transmission to humans. In consequence it was believed that the bovine form is "older" and thereby potentially less virulent to humans. This, however, is not consistent with current clinical observations.

First doubt whether this hypothesis is correct or not came from a recent comparative genomic analysis of various strains of pathogenic mycobacteria. Accordingly, Brosch et al. [3] compared distinct gene segments of currently available mycobacteria of the MTB-complex and suggested that the presence/absence of these segments were due to evolutionary development. The comparative analysis hypothesized that a human strain of mycobacteria, *M. tuberculosis*, represents the "most ancient" strain. During the later development the other pathogenic mycobacteria split off including the bovine type (Figure 1). This intriguing hypothesis, however, can provide neither any time frame nor any spatial distribution of the different developmental stages in the history of mankind. As well no data can be deduced to indicate any potential environmental influence on mycobacterial evolution.

Accordingly, only the direct molecular analysis of ancient human (and animal) biomaterial is able to solve these questions. Furthermore, such a molecular analysis may also answer two other major questions: (i) was human tuberculosis a rare or a frequent disease in ancient populations? and (ii) which strain(s) occurred at which time period in ancient populations? The answers may also provide a basis for understanding the influence of any environmental factor on mycobacterial evolution.

3. The Pathomorphology of Osseous Tuberculosis

In its very typical form osseous tuberculosis is a chronic destructive inflammatory process with tubercle formation in bone comparable to affection of other organs/tissues. The predilection sites of osseous tuberculosis are not only the (thoracic and lumbar) spine and the epiphyses of large joints, but also the skull (tuberculose meningitis) and various small and flat bones. In general, every bone may be affected. The spinal affection seems to result from a spread from the thorax (pulmonary and pleuritis tuberculosa) to the adjacent vertebral bodies (via lymphatics and the paravertebral venous plexus) or the systemic spread by the blood stream. The latter may explain the higher frequency of tuberculose arthritis and the involvement of the epiphyses.

The end stage of tuberculosis of the spine is the very typical ventral collapse of the vertebral body leading to a more or less severe angulation of the vertebral column ("gibbus") (Figure 2). This condition indicates long-standing bone tuberculosis; the lesions are typically characterized by severe alteration of the ventral side of the vertebral body and the presence of fistulas of the bone and/or the intervertebral disc. Major differential diagnoses comprise trauma sequels and some other spinal infections, such as brucellosis. While trauma-induced defects mostly affect the ventral and dorsal sides of the vertebral body, brucellosis does not show fistular defects.

Recently, we [4] have identified a considerable number of less typical osseous lesions, particularly of the spine, which proved molecularly positive for the MTB-complex. These lesions are frequently characterized by an irregular pitting of the ventral vertebral body, but lack of vertebral collapse and formation of fistulae (Figure 3). The differential diagnosis of those lesions includes trauma sequels and a variety of different infectious diseases, including mycobacterioses. Previous molecular studies on those "nonspecific" vertebral suggest that mycobacterial infection may represent a major part of those lesions [4, 5].

4. First Evidence for Tuberculosis in Human History

The presence of tuberculous infections in human history was first evidenced by very typical macroscopic changes of infected bones, mainly of spinal lesions (see Section 3), which have been found since the Neolithic period (i.e., approx. 3000–7000 BC). One of the earliest observations of typical spinal tuberculosis in Europe comes from the cave of Arena Candide (Liguria) in Italy where the material was dated to the first half of the fourth millennium BC [6]. Slightly "younger" are cases of comparable morphology from Denmark [7] and Germany [8]. Very recently, a combined morphological and molecular investigation on a Neolithic finding from the Mediterranean costal region of Israel described two individuals, mother and child, presenting with suspicious bone pathology for TB which was confirmed by molecular analysis (see Section 8). These two

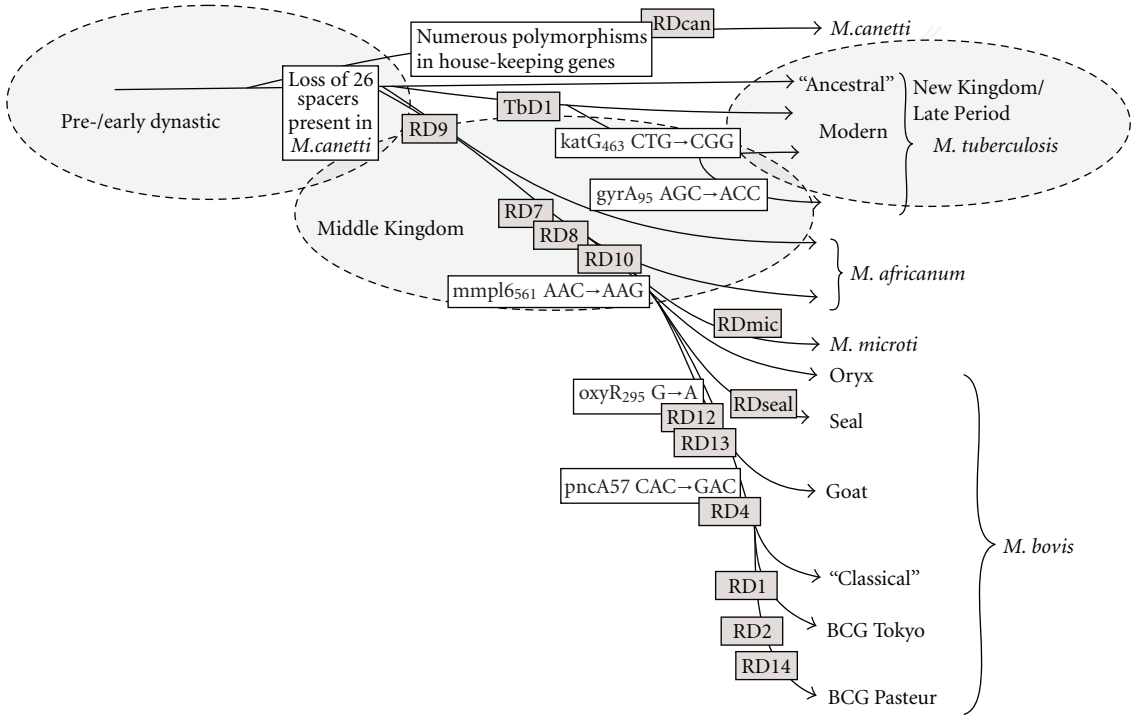
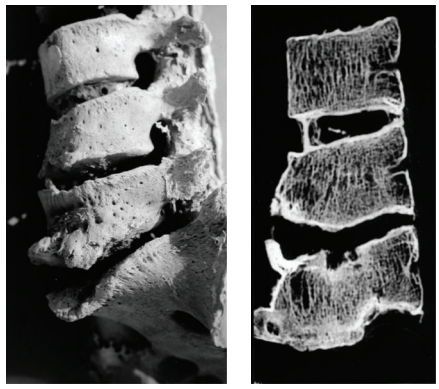


Figure 1: *Schematic diagram of the potential evolution of the various tuberculosis strains (modified according to Brosch et al. [3]) and its impact on ancient Egyptian findings*. The circles indicate the presumed location of the TB main strains as identified by spoligotyping and typical mutations in the various ancient Egyptian populations.



(a) (b)

Figure 2: Typical macrophology of spinal tuberculosis with (a) ventral destruction of the affected vertebral bodies and (b) fistular defects of the bone.

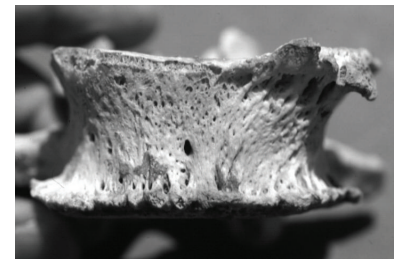


Figure 3: Macromorphology of nonsepcific pathological alteration of the ventral side of the vertebral body, which is still suggestive for a very early phase of osseous tuberculosis.

cases date back to approximately 7000 BC [9]. Significantly more cases have been identified on the basis of the spinal morphology in the Roman Period and the Middle Ages. Currently, it is believed that the number of tuberculosis infections here generally increased in the period following the growth of the population—especially in the expanding townships—following the hunter and gatherer populations of the Neolithic period. However, there exists as yet no proof for that assumption. Most of these observations are based merely on morphological analysis, but still await molecular confirmation. Furthermore, some data base has been gathered from skeletal analysis of some populations from Middle Europe (Germany, Hungary, Latvia, France) from the time periods between Middle Ages and recent centuries.

In contrast to Europe, much more information is available for those regions where accidental or intended mummification occurred, especially in South America and Egypt. Here again, typical morphological lesions of bone and/or residual soft tissue indicated the presence of human tuberculosis since early periods (c. 3500 BC). While only few cases of the Pre-Columbian mummies have as yet been analyzed by molecular techniques providing a successful amplification of tuberculosis ancient DNA [10–13], much

more data are available for the Egyptian material [5, 14–18]. Therefore, it makes sense to have a closer look at the current data-base in the Egyptian material. Unfortunately, as yet no successful molecular investigation has dealt with populations having a hunter-gatherer lifestyle, predating animal domestication and agriculture. The "oldest" case of molecularly proven infection with mycobacteria goes back to a c. 17,000 years old bison [19], but no human infection that is older than 9,000 years before present has been successfully analyzed.

5. Evidence for Human Tuberculosis in Ancient Egypt

The first evidence that human tuberculosis was present in ancient Egypt came also from typical macroscopic osseous changes in human remains, which were in this instance the well-preserved Egyptian mummies. Likewise, one of the first "cases" has already been described in 1910 [20]. The mummy of the Amun priest Nesperhan presented with typical ventral destruction of the lower thoracic spine leading to the typical gibbus formation of spinal tuberculosis (see above). Additionally, ossified paraspinal abscesses along the paravertebral muscles were present in this mummy. Besides this very typical case some other samples of spinal lesions were detected. In a previous compilation, a British group [21] was able to summarize 31 cases of presumed spinal tuberculosis in published material from ancient Egypt.

In 1997 our study group was the first that identified ancient DNA fragments specific for the human *M. tuberculosis* complex in a mummy with typical morphological signs for spinal tuberculosis [14]. Due to this case, we were able to improve the analytical technology allowing to analyze the molecular characteristics of ancient human tuberculosis.

6. The Molecular Analysis of Human Tuberculosis—Technical Prerequisites and Avoidance of Contamination

The analysis of ancient DNA (aDNA) is always risky with respect to contamination with recent DNA, since the first is fragmented and mostly very poorly preserved, while the latter is well preserved and therefore much easier amplified/analyzed. Therefore, even very minor amounts of recent DNA can produce artefacts. In order to minimize the risk of contamination a series of conditions have been claimed and all studies from our own laboratory—as well as other published material—have to meet those criteria. These include: (i) whenever possible, removal of samples on-site; (ii) storage of samples in sterile lab ware; (iii) all lab work only in rooms that are exclusively dedicated to aDNA analysis; (iv) removal of the sample surface (by chemical and/or mechanical treatment); (v) wherever possible, use of single-use lab ware; (vi) sterile bench conditions and extensive precautions for the lab personal to avoid carry over of material; (vii) replication of data in a second, locally distant laboratory.

The criteria for the work with human aDNA have to be kept extraordinarily strict, which seems to be less at risk for the work with mycobacterial aDNA since the modern day contamination sources are restricted to laboratory carry-over effects (therefore, use of rooms dedicated only to aDNA analysis), but less to persons that might be infected with the bacteria. In all our own studies—that are part of the present review—those criteria have been strictly obeyed. Furthermore, an additional "control" for the mycobacterial analysis is presented by the MTB-strain analysis (e.g., by spoligotyping) which might have rapidly detected contamination with specific (recent) strains. Similarly, a carry-over contamination of the pre-PCR material with post-PCR fragments is (at least in those studies performed by our own group) not very likely since in that case identical spoligotyping patterns of several cases should have been expected. This was not the case in our studies. (In other studies, such as that by Hershkovitz et al. [9], the spoligotyping provided only incomplete and not sufficiently conclusive patterns which may be due to less optimal preservation of the material than in the "younger"—and artificially mummified—Egyptian biomaterial). Although we have accordingly no evidence that the contamination problem affected most data shown in this compilation, it cannot be fully ruled out that ancient contaminations occurred that might have influenced any observation.

7. Molecular Estimation of Tuberculosis Frequency in Various Ancient Egyptian Populations

Meanwhile our study group has analyzed 160 samples—mostly bone samples of the spine—for the molecular presence of tuberculosis. This material came from two sites, the pre- to early dynastic necropolis of Abydos, Upper Egypt (c. 3200–2800 BC), and several tombs of the "Necropolis of the Nobles" of Thebes-West which cover a time period from the so-called "Middle Kingdom" (2000–1600 BC) and the "New Kingdom" (1600–1000 BC) until the "Late Period" (800–500 BC). Out of this material three major time periods could be evaluated separately: the pre- until early dynastic period ($n = 13$), a tomb exclusively used in the Middle Kingdom period ($n = 45$) and several tombs built in the New Kingdom and used until the Late Period ($n = 102$). In this material we detected in 4 of the 13 pre- to early dynastic cases analyzed, in 13 of 45 samples of the Middle Kingdom, and in 16 of 102 New Kingdom until Late Period samples specific mycobacterial ancient DNA of the tuberculosis complex (Table 1).

Although there are differences in the preservation status of the biomaterial between the predynastic period until the Late Period (mainly due to advancing techniques of mummification/conservation), these studies reveal very similar tuberculosis frequencies of approximately 30% (pre-/early dynastic), 29% (Middle Kingdom), and approximately 20% (New Kingdom until Late Period) (differences statistically not significant) suggesting comparable infection rates in the

Table 1: Molecular paleoepidemiology of tuberculosis in various historic populations.

Localization (country)	Period/dating	Morpho-logically typical cases* (*n*)	Morpho-logically suspective cases* (*n*)	Molecu-larly analyzed cases (*n*)	TB-pos/typical + suspective cases (*n*)	TB-pos./insuspective cases
Abydos (Egypt)[A,B,D]	3000–2500 BC	2	6/189	13	2/8 (25.0%)	2/5 (40.0%)
Thebes (Egypt)[B,D]	2000–1600 BC	1	12/211	45	12/13 (92.3%)	1/32 (3.1%)
Thebes (Egypt)[A,D]	2000–500 BC	3	30/226	56	3/33 (9.1%)	0/23 (0%)
Thebes (Egypt)[A,D]	1600–500 BC	5	18/519	46	13/23 (56.5%)	5/23 (21.7%)
La Celle (France)[E]	500–1200 AD	2	9/105	11	5/11 (45.5%)	n.a.
Bacsalmas (Hungary)[F;G]	1600–1700 AD	12	18/480	46	12/30 (40.0%)	2/16 (12.5%)
Rain/Lech (Germany)[C]	1400–1800 AD	11	48/2.547	59	10/59 (17.0%)	n.a.
Sulzbürg (Germany)[H]	1550–1750 AD	0	0/25	25	0	3/25 (12.5%)

*: Typical TB cases and cases with macropathology that might represent mycobacterial disease. For the morphological characteristics of "typical" and "suspective" cases see the text. All data have been taken from own previous publications (some with recalculation in order to render data comparable) ([5][A], [15, 16][B], [17][C], [18][D], [22][E], [23][F], [4][G], [24][H]).

various populations of ancient Egypt over a time period of almost 2500 years.

It has, however, to be taken into consideration that (i) the overall numbers of samples of these studies are still very low so that any generalization must be made with great care and (ii) we initially selected samples for their morphological appearance suggestive for osseous tuberculosis. Thereby, at least at the first glance a certain selection bias was introduced into our analysis which, however, was not different between the various subpopulations so that those data may be comparable between each others, although only to limited extent to other molecular studies. Accordingly, we also analyzed the results of tuberculosis frequencies with respect to the morphological appearance of osseous lesions. In more than 75% of those cases with the very typical morphological features of tuberculosis (e.g., typical spinal "gibbus", Figure 2) the molecular analysis provided a positive result. Even in those cases with slight, but nonspecific osseous lesions (see above, Figure 3) even 20% of cases were tested positive, so that an overall estimate of approximately 40% of typical/suspicious cases was positive for TB aDNA (Table 1). Most surprisingly, almost 10% of bone samples with unremarkable morphology provided a positive molecular result. The latter result is explained by the fact that pulmonary tuberculosis may lead to a systemic spread with the blood stream even without forming typical morphological lesions, for example, in the ultimate premortal time period. This has been confirmed by a recent own study including a series of recent autopsy cases with autoptical evidence for "systemic" spread (in this case: lymph node affection beyond the most intimate lymph node of the primary complex, miliar tuberculosis, or Landouzy sepsis) showing a high percentage of molecularly positive results in otherwise morphologically unremarkable vertebral bodies. In consequence, the molecular identification of *M. tuberculosis* complex aDNA suggests a premortal systemic spread of the bacteria.

In addition, those various "study populations" represent interesting models to calculate the rough infection rates in ancient populations, such as those from ancient Egypt. Supposing a comparable frequency of macromorphologically detectable affection of bone by systemically spreading TB (in present day populations approx. 5%, McTammany et al. [25]), the macroscopic identification of 12 typical cases in c. 1.000 mummies and skeletons (predynastic until Late Period) indicates a tuberculosis prevalence of approximately 25% of the populations. Unfortunately, the distribution of "typical TB cases" is not even in the different time periods analyzed and some populations even do not show those typical cases so that differences in the TB infection rates cannot be deduced until now.

8. Molecular Analysis of Tuberculosis in Other Historic Populations

Meanwhile, several laboratories have successfully identified tuberculosis DNA in a broad range of populations from differing time periods, for example, Neolithic Israel [9], Pre-Columbian Peru [10] and Northern Chile [11–13], Byzantine Turkey, Pre-European contact Borneo and Romano-British England [26], Medieval England [27, 28], Medieval Lithuania [29], and 18th-19th century Hungary [30]. Our study group contributed studies on Medieval Hungary [4, 23], Southern Germany [17, 24], and Southern France [22].

Out of these studies only few reports cover a series of cases, most studies cover still case reports or summarize few isolated cases without providing a population basis. Beyond this, the analysis of a Lithuanian series [29] describes tuberculosis infection on a population basis with approximately 25% tuberculosis prevalence which is very comparable to a series of natural mummies from Christian Nubia [31]. Similarly, observations on a Middle Age population from Southern Hungary (Avar period, c. 700–900 AD, Bacsalmas, Hungary) have been done on a total of 46 out of 480 cases [23]. Again, we detected a TB frequency with approximately 30% which is in the range of the Egyptian material (Table 1).

A recent molecular study on several Hungarian natural mummies (AD 1731–1838) revealed in 55% of that population ($n = 168$ individuals investigated) a positive result for tuberculosis [30, 32]. There was a greater proportion of

tuberculosis positive cases in mature adults than in senile and adolescent ones. Due to the excellent conservation the successful amplification of ancient DNA in this population was exceptionally well.

Further, but mostly small studies on samples from Southern France (La Celle, Provence [22] and Southern Germany (Rain/Lech; [17], and Sulzbürg [24] reveal comparable infection rates as in the other series suggesting the presence of mycobacterial DNA in human populations dating back to approximately 3200 BC at a surprisingly high prevalence level for tuberculosis infections with comparably high numbers.

In summary, all studies using the molecular analysis of identification of *M. tuberculosis* in historic populations between 3.200 BC and 1800 AD suggest constantly high infection rates with an increase only in the last few centuries (e.g., Hungary, which might be significantly influenced by the excellent preservation of the Hungarian mummies). In terms of influence of environmental factors, therefore, the significant differences like, for example, in climate, nourishment, or social stratification obviously does not influence the infection rates of this disease. However, since all populations investigated as yet belong to populations in settlements, this may be responsible for the high prevalence of TB.

9. Molecular Identification of Tuberculosis Strains in Ancient Egyptian Material

Besides the molecular identification of presence or absence of human-pathogenic mycobacterial DNA, the analysis of the tuberculosis strains is of utmost interest. This especially is necessary to evaluate evolutionary aspects of the mycobacteria and to identify the spatial and temporal distribution of the infections in various human populations. At present data on the mycobacterial strain distribution are available for ancient Egypt [15, 16], prehistoric Siberia [33], Medieval Britain [34], and the Hungarian mummy population (see above [30, 32]). The data were obtained either by the analysis of strain-specific gene segments of the human pathogenic mycobacterial strains (e.g., genes that occur only in one strain or genes that show specific gene variance at specific spots) or by a special amplification procedure where the presence/absence of spacer regions of the IS6110-gene is determined. The latter procedure, termed "spoligotyping", provides a typical signature of spacers that is unique to different mycobacteria strains. It is of note that both approaches show less high numbers of successful amplifications as compared to the usually investigated multicopy IS6110-gene (the marker for presence/absence of tuberculosis DNA) since the "drop-out" of gene regions affects these regions more easily than that of the multicopy gene IS6110 in total. Therefore, not every case with successful IS6110-amplification also shows a positive mycobacterial strain identification.

In the "oldest" material from ancient Egypt [18], only few successful spoligotyping results could be obtained from the pre- until early dynastic material (Abydos, c. 3200–2800 BC). Accordingly, we were able to obtain 2 complete and 4 incomplete spoligotyping patterns in the pre-/early dynastic material. However, more data are available for the Middle Kingdom (c. 2000–1600 BC) and the New Kingdom until Late Period (c. 1500–500 BC). The resulting data showed a significant variance of strains with those that are at present widespread over the whole world and those that are restricted to specific areas. Interestingly, in none of the as yet successfully amplified 160 cases *M. bovis* was present. To this respect, one has to take into consideration that *M. bovis* may have escaped much more easily the molecular analysis than *M. tuberculosis*, since *M. bovis* contains fewer copies of the IS6110 sequence than *M. tuberculosis*. Furthermore, the pre- to early dynastic material revealed an "old" strain of *M. tuberculosis (typus humanus)*, no mutations in den katG and gyrA genes and no deletions of TbD1 and RD9; $n = 2$ [18], which is slightly different from the widespread "modern" *M. tuberculosis* strain in spoligotyping (mutations and deletions present), the Middle Kingdom material additionally *M. africanum* strains, and the New Kingdom until Late Period material a "modern" strain of *M. tuberculosis (typus humanus)*, but not the "old" strain seen in the pre- to early dynastic period. Since these observations are coming from different tombs (and two different burial sites) we cannot conclude a direct evolutionary line. However, the data are very well in agreement with the hypothetical assumptions by the Institute Pasteur group [3, Figure 1] suggesting that a "switch" between "old" to "modern" *M. tuberculosis (typus humanus)* occurred between c. 2000 and 1500 BC. *M. africanum* is also seen in a very early population so that its role in early evolutionary processes is plausible (possibly representing an evolutionary parallel development to the *M. tuberculosis*). Finally, it is of highest interest that *M. bovis* is (at least until now) not seen in any ancient Egyptian sample analyzed up to now.

Interestingly, the as-yet oldest molecularly proven human case of mycobacterial infection [9] showed at least some features of a "modern" pattern of mycobacteria. Thus, the authors were able to identify a TbD1 deletion. In consequence, the aforementioned "evolutionary scheme" may be subjected to differences in the time scales of various regions and/or populations. At present, the number of molecularly investigated cases is too small to allow final conclusion and eventual surprises are not excluded.

However, well in line with all the observations, the Medieval British and the Hungarian mummy populations revealed only *M. tuberculosis (typus humanus)* strains, but not *M. bovis*. Here, no *M. africanum* was detectable. However, very recently, Taylor et al. [33] described four cases of *M. bovis* infection in skeleton from a Siberian Iron age cemetery (dating back c. 2000 years BP) suggesting that this strain originated in the East with more recent spread to the West. To this respect, it has to be investigated not only whether special features of environment, such as distinct living conditions, contact between animals and humans, but also whether climate may have been influential factors for the development of the *M. bovis* strain and the subsequent infection of humans.

10. Possible Evolutionary Scenario from Theoretical and Paleomicrobiological Analyses

The growing puzzle of data offers new insight into the distribution and development of human pathogenic mycobacteria. We thereby suggest that an "old" strain of the human type of tubercle bacteria was (at least one of) the first original strain. It is still plausible that this was acquired from animal source, be it of caprine or bovine origin. This question, however, remains open until adequate animal material is investigated. There exists at least one hint for this hypothesis: in a case study Rothschild et al. [19] reported *M. tuberculosis* DNA in bones from a 17,000 years old bison. The genotyping of this observation furthermore suggests a genotype which is closer to *M. tuberculosis* (human type) than to any other mycobacteria. In consequence, the analysis of animal residues originating from the period of early domestication is of particular interest to substantiate this issue.

In addition, our identification of *M. africanum* in a tomb complex that had exclusively been used in the Middle Kingdom, as well as in a further Middle-New Kingdom tomb from Thebes-West, is also of particular note since this type of mycobacteria is assumed to originate from Central/Eastern Africa and a transmission to Egypt in the indicated time period of intense trading connections to the Sudan area is plausible.

Our data of the New Kingdom until Late Period material shows a dominance of "modern" type of *M. tuberculosis* strains, such as those that dominate the present day isolates affecting humans. Accordingly, we have good evidence that the "transition" from "old" to "modern" *M. tuberculosis*—already suggested by Brosch et al. [3] on a hypothetical basis—indeed happened and that this may have occurred between the early dynastic to New Kingdom period, that is, in a time frame of about 1500 years. Further analysis may narrow this frame even more (see also Figure 1).

11. The Climate in the Historic Nile Valley

Without the river Nile, Egypt would be (and have been) a complete desert region (except for the coastal areas). The unique geographical condition of a several thousand kilometres long "river oasis" was fundamental for the origin of the ancient Egyptian empire and its persistence for several millennia. Further to the mere transport of water into a completely arid region (with less than 5 mm precipitation/m^2 per year in the South) the river Nile provided the country with an extremely fertile soil. Since the yearly Nile flood was so essential for the ancient Egyptians, the level of the river was precisely recorded. Dating back to the year 622 AD we have fairly precise measures of Nile water gauges, further, though incomplete information goes back to c. 3.000 BC. This is an important source of information for the paleoclimate of the region. Further data come from comparative investigations in Europe (e.g., glaciological analyses of Alpine glaciers) and the stable isotope spectra obtained from human remains from various time periods of ancient Egypt. Although these datasets result from very different approaches, they provide us with a rather rough, but interesting climatic pattern in pharaonic Egypt.

Especially the observations of the so-called "nilometer" at the island of Elephantine (Assuan) at the southern entry point into ancient Egypt are of significant use. Likewise Hassan [35] and Riehl and Meitín [36] provide us with a precise change in the Nile floods between c. 620 AD and 1950 AD showing short-period climate changes of the Nile River. These studies clearly showed 8 events of climate variation with duration of ca. 100 years each which was temporarily interrupted during the "little climatic optimum" between c. 1300 AD and 1600 AD. Similar changes have been deduced from the record of the Palermo Stone that indicates similar climatic changes going back to 3000 BC [37]. These data—together with sediment analysis of the Lake Qarun in the Fayum depression which is fed by annual overflows of the Nile—indicate a significant climate change in predynastic ancient Egypt at c. 4200 BC, 3800 BC, and 3000 BC. Resulting prolonged aridity was also determined by approximatly 1 m lower annual Nile floods [35] causing persistent drought in Egypt. This must have significantly influenced daily life in ancient Egypt.

The second set of evidence comes from glaciological studies in the Alpine region where the investigations provide evidence for a cooler and relatively rainy period in the Near Eastern (Ugarit, Syria) [38] along with sediment changes in the Euphrates River at that period. Although these data do not directly represent the Nile region, it seems fair to assume that the climate change during that period may have also influenced the Northern part of Egypt as well (Lower Egypt).

Finally, the stable isotope pattern of human remains from predynastic (c. 3200–3000 BC) to First Intermediate Period (i.e., c. 2200 BC) and Late Period to Roman findings (i.e., c. 500–30 BC) also suggests climatic changes in ancient Egypt [39]. These investigations used the pattern of stable oxygen isotopes to find out that the paleoclimate in the predynastic period was moister than in the period between the Old Kingdom and the Middle Kingdom (the so-called First Intermediate Period, c. 2200–2100 BC) and also the Late until Roman Period. The findings suggest that there was a temperature decrease from predynastic to First Intermediate Period and a temperature increase until the Late to Roman Period.

Taking these various pieces of (mostly indirect) evidence for the paleoclimate of ancient Egypt together we can identify cyclic minor climate changes along with a more significant worsening of the climate very early and an amelioration of the climatic conditions later.

Due to its central significance, the environmental conditions are essential factors for the evolution of all organisms. This holds particularly true for bacteria that live under a severe selection pressure in terms of host-pathogen interaction and virulence. To this respect the climate, that is, particularly environmental temperature, humidity, and so forth, plays a central role. Although this role and its interaction have not been systematically investigated our very preliminary data (on a very restricted study population) provide us with some beginning insight into the interaction

of climate conditions and pathogens. With the advent of molecular techniques and the direct analysis of the traits of pathogenic bacteria in human remains from historic populations, we have now the opportunity to broaden our insight into the interaction of "climate" and bacterial evolution. Due to the technical requirements up to now only very few pathogenic bacteria and small series of samples have been analyzed with a central focus on mycobacteria, that is, human tuberculosis. Nevertheless, this unique approach offers us first interesting observations.

(i) Several studies on the human remains from various time periods and regions (mostly in Northern Africa and Central Europe) suggest constantly high prevalence rates in the study populations.

(ii) Due to the significantly different environmental conditions both of the host and the pathogen (e.g., in terms of "climate"), this suggests that the virulence of the mycobacteria was not significantly influenced by any environmental change—at least since the living habitats of ancient populations were not considerably different. This, however, also means that changes in the living mode, for example, changes from hunter-gatherer cultures to township populations, may be a much more central issue with diseases development and bacterial evolution.

(iii) There are some very interesting aspects of strain development of the *M. tuberculosis* complex which are linked to distinct time periods and regions. A closer analysis of the concomitant environmental factors will be an important issue to identify modulatory conditions.

In summary, although we are at the very beginning of understanding the evolution of bacteria and their interactions between hosts and environment, the paleopathological approach seems to be a highly interesting one also in terms of finding out what conditions have positive or negative influence on distinct evolutionary pathways. Hopefully, the advances in paleopathological techniques will enable us to answer these questions.

12. Perspectives

Recent advances in the molecular detection and characterization of microbes in ancient tissue samples provide a new insight into the distribution and evolution of distinct pathogens during human history. Thereby, the direct paleomicrobiological analysis of human remains provides a more and more precise picture of human tuberculosis and the underlying mycobacterial strains. Preliminary studies confirm that modern PCR techniques are capable directly analyzing this issue. Furthermore, it is becoming clear that recent theoretical models of mycobacterial evolution—based on strain differences of recent isolates—indeed occurred in history. Although the picture provides still some isolated and not well linked pieces of puzzle, we suggest an origin of *M. tuberculosis* strains ("old" strains) in the dawn of civilization, but not that of *M. bovis* as previously assumed. Possibly originating from "old" strains, some modern types of *M. tuberculosis* evolved—with some differences in the local and spatial distribution over time and various regions. Ongoing and future paleopathological and paleomicrobiological investigations will hopefully contribute to this picture.

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Review Article

Climate Change and Malaria in Canada: A Systems Approach

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This article examines the potential for changes in imported and autochthonous malaria incidence in Canada as a consequence of climate change. Drawing on a systems framework, we qualitatively characterize and assess the potential direct and indirect impact of climate change on malaria in Canada within the context of other concurrent ecological and social trends. Competent malaria vectors currently exist in southern Canada, including within this range several major urban centres, and conditions here have historically supported endemic malaria transmission. Climate change will increase the occurrence of temperature conditions suitable for malaria transmission in Canada, which, combined with trends in international travel, immigration, drug resistance, and inexperience in both clinical and laboratory diagnosis, may increase malaria incidence in Canada and permit sporadic autochthonous cases. This conclusion challenges the general assumption of negligible malaria risk in Canada with climate change.

1. Introduction

Canada's climate could support, and has supported, local malaria transmission in the past. Malaria was introduced to continental North America in the 16th and 17th centuries by European colonists and African slaves [1]. The disease spread with settlement until being controlled in the early 1900s and eradicated in the 1950s [1]. Extensive debate and research has focused on the potential for climate change to alter or increase malaria distributions and incidence both globally and regionally. Global models and research discourse focuses—justifiably—on the impact of climate change on the spread and magnitude of global endemic malaria in at-risk regions and regions at the margins of current endemic distributions. Changes in malaria incidence in Canada and similar northern countries are assumed to be negligible. In this paper, we challenge this assumption.

The 4th assessment report (FAR) of the Intergovernmental Panel on Climate Change (IPCC) highlights, with increased confidence, the projected impact of climatic change on infectious diseases and human health [2]. These projections are supported by a growing body of literature which has contributed to the characterization and quantification of climate as a determinant of disease distributions and incidence [3–10]. The assessment of climate impacts on infectious diseases, however, is challenged by often complex interactions of climatic determinants with other ecological, economic, and social determinants of disease incidence [5, 6, 9, 10]. This has led to efforts to quantify the disease burden that is specifically attributable to climate change [4, 8], but disentangling the causal contribution of anthropogenic climate change from complex disease systems poses both analytical and conceptual difficulties. Analytically, it is often difficult to characterize and quantify causes of disease variation in time and space, and to quantify these while controlling for variation in nonclimatic determinants. Furthermore, both the direct and indirect effects of climate change are likely to impact vector-borne disease occurrence;

Table 1: Common factors in systems approaches to environment and health.

1. Explicit integration or consideration of the following in analyses:
(i) Multiple disciplinary perspectives (e.g., human and biophysical environments)
(ii) Nonproximal or qualitative factors affecting transmission (e.g., technology, economic development, public health measures)
(iii) Processes acting within, and across, multiple spatial, and temporal scales
(iv) Interactions, synergisms, and nonlinearity
2. Use of concept maps (or visual systems graphics) to frame and guide analyses

for example, extreme weather events (or indeed long-term changes in climate) may result in population migration and subsequent changes in population health and exposure. Increasing sophistication in transmission and systems modeling, however, has provided innovative approaches to confront this challenge [5, 7, 9]. The IPCC FAR, for example, does itself integrate social and ecological determinants of disease [3].

Within the climate change and health literature, there have been parallel developments in the application and development of systems frameworks for assessing environmental change impacts on infectious disease [6, 10–13]. These approaches arise from a range of (inter)disciplinary and pedagogical roots, including environmental health, social epidemiology, environmental change, and systems theory [13]; the emergence of conceptual systems frameworks has included eco-epidemiology [14], ecohealth [15, 16], social epidemiology [17], and vulnerability science [18, 19]. Ecosystem health approaches, for example, have been widely used to provide new and integrative frameworks for conceptualizing, understanding, and characterizing complex dynamics within health systems [6, 20–23]. These approaches draw on general and complex systems theory [24–27] to conceptualize health problems as highly integrative systems affected by interacting processes of social and ecological complexity. The theoretical basis of these approaches is that understanding complex systems can only be achieved by looking at how different parts of a system interact together rather than from teasing them apart [28]. Climate change, in this context, is one of several determinants of infectious disease occurrence, whose impact is superimposed upon, and moderated by, parallel changes in nonclimatic determinants. The utility of these frameworks is thus not necessarily in isolating the attributable burden of disease due to climate change, but rather in explicitly characterizing the *cumulative* or *integrative* impact of climate change within the context of changes in other disease determinants. Such frameworks are particularly useful for preliminary characterization and identification of key processes, interactions, scales, and feedbacks—an exercise which can inform and guide integrative attribution modeling [12, 13, 20].

In this paper, we use a systems approach to begin to assess how climate change, by having multiple proximal and distal influences on determinants of transmission, might affect the occurrence of malaria in Canada. It has been generally concluded that there is negligible risk of malaria in Canada and similar northern countries due to climate change [27–29]. This assessment has largely been based on the assumption that the importance of malaria in these countries rests solely on the likelihood of endemic malaria becoming established. In this paper, we challenge this assumption, drawing on four key premises. (1) Absence of risk of *endemic* malaria does not preclude the potential for changes in incidence due to imported or sporadic autochthonous (i.e., locally acquired) malaria. (2) Even small changes in malarial incidence or emergence of autochthonous cases in Canada and other nonendemic countries could have important implications for public health systems. (3) Current models of climate impacts on malaria are not designed to effectively or accurately evaluate the potential for changing malaria incidence in peripheral regions and should not be interpreted as such. (4) Systems approaches provide a useful framework for conceptually and methodologically integrating social and biophysical determinants of disease.

We begin the paper by describing the methods used in the study, before reviewing the nature of malaria incidence in Canada. We then assess how climate-malaria links have been approached in the literature in general and argue that assumptions of limited risk in Canada are not supported by existing climate-malaria research, and merit re-examination in the context of sporadic incidence and the role of nonclimatic determinants. The paper then explores how climate change might affect malaria incidence in Canada based on an understanding of the malaria transmission cycle and its climatic determinants. Using a systems approach, we assess how nonclimatic determinants of malaria risk may exacerbate or moderate climate-related changes in malaria incidence. The characterization presented here represents a preliminary qualitative review and synthesis of existing knowledge and literature and should therefore be considered descriptive and exploratory.

2. Methods and Approach

A summary of common themes and considerations used in systems frameworks was developed to guide this review (Table 1). A systems graphic was developed by adapting the malaria life cycle model to identify and include both proximal and distal transmission determinants. Key parameters were reviewed and assessed with respect to trends, interactions, and potential impact on transmission. We performed an integrative review and analysis of existing literature and data related to malaria transmission and dynamics, Canadian malariology research, climate change science, projections for Canadian climate, demographic trends, international travel,

and current models and research related to both global and regional impacts of climate change on malaria transmission.

Canadian malaria data were acquired from the Public Health Agency of Canada Notifiable Diseases registry [29]. Distributions of mosquito vectors were reviewed to identify competent vectors with distributions in Canada and with the highest vector potential for parasite transmission [1, 30–37]. Existing records of autochthonous malaria cases were reviewed, recorded, and combined to map autochthonous malaria in Canada and the United States (1957–2003) [1, 38–45]. These cases were overlaid with key vector distributions using ArcMap (ArcInfo 9.2, Environmental Systems Research Institute, Redlands, Calif, USA).

Temperature data were compared to parasite development thresholds and dynamics using three sources of data: effects of temperature on the sporogonic cycle of *Plasmodium* spp. in mosquitoes, Canadian climate data, and downscaled climate change projections. The effects of temperature on the duration of development of the parasites in the mosquito were as follows: *P. vivax* requires approximately 30 days at 18°C or 20 days at 20°C, while *P. falciparum* requires approximately 30 days at 20°C. Above 33°C or below 16/18°C (for *P. vivax* and *P. falciparum*, resp.), the cycle cannot be completed and transmission cannot occur [1, 46]. Data on the maximum number of consecutive days >18°C in Toronto (1970–2006) were calculated using archived Environment Canada climate data [46]. Data from Toronto were selected since Toronto is the largest Canadian city within the distributional range of a competent malaria vector. Downscaled climate change projection data were used to characterize projected temperature changes on transmission potential. The climate change projections were obtained from interpolation (for Chatham, Ontario) of output from the CGCM2 (Canadian Coupled Global Climate Model 2) [47] that incorporated estimated forcing calculated in emission scenario forcing "A2" [48]. The output was downscaled using LARS-WG stochastic weather generator. LARS-WG was calibrated with 30 years of daily weather observations at Chatham (and its predecessors) obtained from the Environment Canada database. Chatham was selected because it is the location closest to Toronto for which we already have downscaled projections. The data and methodology used here are described in detail by Ogden et al. [49].

3. Malaria in Canada and USA

As recently as the 1820–30s, Canada experienced malaria epidemics, including severe outbreaks during construction of the Rideau Canal in eastern Ontario, and outbreaks in Montreal and the prairies [50]. Records suggest endemic malaria occurred in the mid to late 1800s on the shores of Lake Ontario from Kingston to Hamilton, along the northern shore of Lake Erie, along the whole St. Lawrence River and its tributaries, in parts of the western provinces, and sporadically in Quebec City and Halifax [36]. Incidence declined steadily in the early 1900s, and its eradication is attributed to increased urbanization and improved socioeconomic conditions which decreased mosquito populations, decreased human contact with mosquitoes, and improved the speed and effectiveness of case treatment [36, 51]. While current socioeconomic conditions have dramatically reduced the risk of local malaria transmission, historical incidence supports the potential for autochthonous malaria in Canada under conditions favouring transmission. For this reason, we evaluate the potential impact of shifts in the climatic and nonclimatic determinants of malaria on the balance of transmission potential.

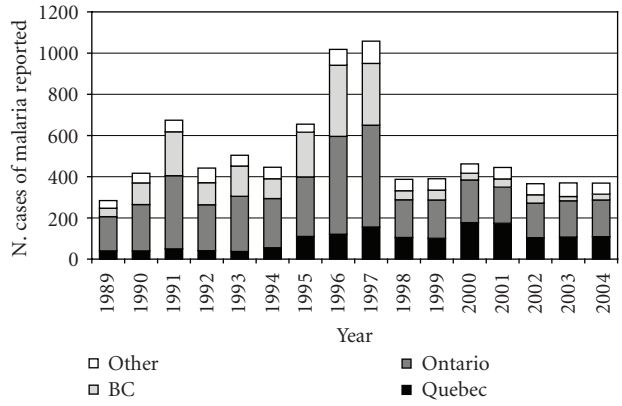

Figure 1: Annual incidence of malaria (caused by all *Plasmodium* species) in Canada. Data obtained from the Public Health Agency of Canada [29].

For malaria transmission to occur, three key factors need to coincide: competent vectors, a suitable climate for transmission cycles (i.e., completion of parasite development in the vector), and infected, infective humans. The first two factors are present in some locations in Canada, and travel and immigration could potentially introduce parasites into local human populations. However, in recent decades these factors have not been sustained at levels sufficiently high or for periods sufficiently long, for transmission cycles to be established, even at a local level. This is because (1) the numbers of infected and infective humans have been too low (due to high standards of living and ready access to medical services and antimalarial chemotherapy) and (2) vector abundance and rates at which mosquitoes could bite humans have not been sufficiently high (due to a combination of climate, water management, housing conditions, and mosquito control). Thus, while malaria transmission occurred in Canada in the 19th century and the requirements for transmission of malaria theoretically exist in Canada, transmission potential since 1900 has been too low to permit local transmission [36, 52–54]. Changes in these and other factors, directly or indirectly associated with climate change, however, have the potential to affect this balance.

An average of over 500 travel-related cases are reported annually in Canada (1989–2004, Figure 1; [29, 55]). This number, which likely represents fewer than half of actual cases [55], is comparable to the incidence of West Nile Virus in Canada in most years (annual average of 459 clinical cases up to 2006 [56])—a disease which has generated widespread media and public health attention in Canada. While the United States records far more cases (>1000/year [57]), reported malaria incidence per capita in Canada is at least

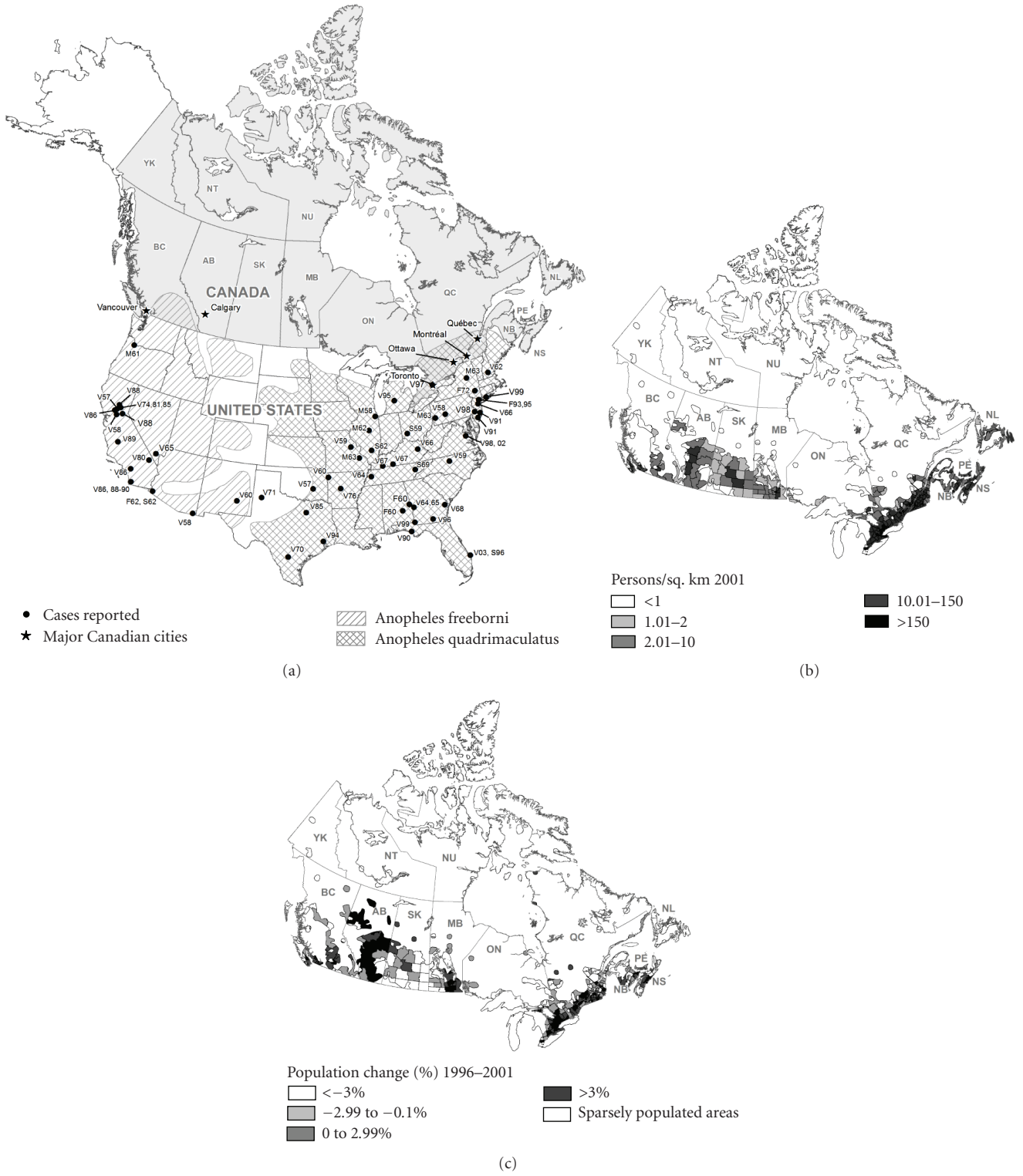

Figure 2: Geographic distribution of vectors of malaria, cases of local mosquito-borne transmission during the period 1957–2003 Figure 2(a), population density Figure 2(b), and population change Figure 2(c) in Canada. (a) shows the geographic distribution of vectors of malaria and cases of local mosquito-borne transmission during the period 1957–2003. Black dots represent location of cases of malaria in the United States and Canada presumed to be acquired from local mosquito-borne transmission between 1957 and 2003 (Source: [1, 38–45]. Each dot represents one or a cluster of cases in a given year. Labels include species type (V = *P. vivax,* F = *P. falciparum*, M = *P. malariae*, S = species unknown) and date. Locations are approximate. Hashed areas represent the approximate distributions of the two most important competent malaria vectors in Canada. (Sources of malaria data: [1, 30–34]). See Table 2 for full names of Canadian provinces. (b) and (c): population density (2001) and population change (1996–2001) in Canada. Source: Population Ecumene Census 2001, GeoGratis, Natural Resources Canada.

Table 2: *Anopheles* species of Canada and their potential competence as malarial vectors.

Mosquito species	Distribution in Canada	Vector competence as a potential reservoir for *Plasmodium* species
An. freeborni	British Columbia (BC)	Competent vector, particularly for *P. vivax,* believed to be the dominant vector of cases in the western USA
An. quadrimaculatus	Ontario (ON) Quebec (QC)	Competent vector for *P. vivax* and *P. falciparum*, believed to be the dominant vector of cases in the eastern USA
An. punctipennis	British Columbia (BC) Manitoba (MB) New Brunswick (NB) Nova Scotia (NS) Ontario (ON) Quebec (QC)	Competent vector for *P. vivax* and *P. falciparum*, but not believed to be dominant vector for human incidence in the USA, possibly due to minimal preference for modern indoor environments
An. walkeri	Manitoba (MB) New Brunswick (NB) Nova Scotia (NS) Ontario (ON) Quebec (QC) Saskatchewan (SK)	Vector competency doubtful; species believed to be of negligible or no importance as a vector of *Plasmodium spp.*
An. barberi	Ontario (ON) Quebec (QC)	Competent vector of *Plasmodium* species, though considered to be of doubtful importance due to its limited contact with man
An. earlei	All provinces & territories except Newfoundland and Labrador (NF)	Not known to be a competent vector

Sources: [31, 35–37].

three times higher than its southern neighbour; the reasons for this difference remain unclear [55, 58]. Nearly, all malaria cases in North America are imported cases brought into the country by people who have become infected while visiting, or after arriving from, an endemic country [59]. Travel and immigration were also associated with two of Canada's most significant recent outbreaks. The first, in 1995–97, involved Canadians traveling to the Indian Punjab region, which was experiencing a *P. vivax* outbreak at that time [59]. The second involved an outbreak in Quebec in 2001-2002 in a population of central and east African refugees recently arrived in Canada from Tanzanian refugee camps [58–60]. While there are few malaria deaths in Canada each year, all malaria cases raise significant community and public health concern [55].

While most cases of malaria in the United States are imported, there have been a number of cases of autocthonous malaria, whereby people with no history of travel to endemic areas have become infected by locally infected mosquitoes. Between 1957 and 2003, there were 156 cases of locally transmitted malaria in US [40]. These cases have not been confined to the southern United States (Figure 2(a)); locally-transmitted cases, for example, have been reported in Virginia, New York, and Michigan [1, 40]. Locally acquired outbreaks are often reported near urban centres or airports, where large influxes of travelers and immigrants are found [40]. Increased air travel, increasing drug resistance, and changing environmental conditions have raised concern over malaria re-emergence in the United States [1]. In Canada, one case of suspected locally-acquired autochthonous malaria was reported in a Toronto woman in 1997 [38, 61] (Figure 2(a)). An unexplained death due to *falciparum* malaria was also recorded in Quebec in 1974, raising questions of local transmission, although this is unlikely give that local climate conditions at that time could not have supported transmission [61, 62].

In Canada, there are six species of *Anopheles* mosquito, of which only two are potentially important vectors of malaria (Table 2). *An. quadrimaculatus*, found in southern Ontario and Quebec, and *An. freeborni* which occurs in south-western British Columbia (BC) (Figure 2(a), Table 2) are considered to be the most important vectors for potential autochthonous transmission in the northern United States, and by extension, Canada [1, 30, 32–34]. Although the regions where these two mosquito species occur represent only a small proportion of Canada's territory, they are located in areas where at least half of Canada's population reside and where the majority of population growth in Canada is occurring [62] (Figures 2(b)-2(c)). The potential population at risk could increase with even small expansions in the geographic ranges of the vectors themselves, and of the areas climatically suitable for parasite transmission. Increased mosquito abundance, duration of seasonal survival, and parasite replication *within existing distribution ranges*, however, would likely affect population risk more than range expansion. There is little information on local or urban mosquito abundance and biting within these ranges; existing mosquito distribution data for Canada (Figure 2(a)) are based on rough estimates dating to the 1970s, with much of the available data more than several decades old [30, 31, 35, 37]. Current, updated, and detailed distributions of competent malaria vectors in Canada remain unknown.

4. Climate Change and Global Malaria

Climatic factors are important determinants of malaria transmission. The parasite can only be transmitted from a mosquito to a human once it has completed a complex cycle of development and multiplication inside the mosquito, called the sporogonic cycle [1]. The length of this cycle (often called the extrinsic incubation period) depends on the parasite species and ambient temperature [1]. Therefore, for mosquitoes to transmit infection from an infected human to an uninfected human, ambient temperatures must be sufficiently high, for a sufficiently prolonged period, (1) for mosquitoes to acquire infection by biting an infected human, (2) for parasites to develop in the mosquito, and then, (3) for the mosquito to bite another human and transmit the parasites. The lifespan of the mosquito is related, among other factors, to air temperature, humidity, and rainfall, which also affect mosquito abundance and the rate at which mosquitoes bite humans [1, 50, 51, 63]. Climate has, therefore, multiple effects on malaria transmission.

Malaria periodicity and outbreaks have long been recognized to be associated with climate and climate fluctuations, particularly, on the fringes of global malaria distribution [64–67]. Global malaria outbreaks have been regularly linked, for example, to heavy rains associated with El-Nino events [64–66]. In the United States, hotter and more humid weather conditions were a common factor in local outbreaks of malaria, including a case at a Michigan campsite in 1995 [1, 68]. These warmer, wetter conditions can increase the survival of the mosquito and reduce the required length of the sporogonic cycle sufficiently to allow the parasite to develop and the mosquito to become infectious where it otherwise would not. Similar localized outbreaks in nonendemic northern countries have also been associated with particularly warm weather [69].

Predictions of global climate change [70] have lead to extensive research interest into its potential impact on malaria incidence [32, 71–79], but how climate change may affect the incidence and distribution of malaria is much debated [80–82]. Differing opinions generally arise from differing conceptual and methodological approaches to malaria modeling. Some research in this area has predicted significant global or regional spread based on biological models that incorporate some climate-driven variables that directly affect the basic reproductive number of malaria (R_0) (particularly parasite replication in the vector); these reflect predictions of extensions in transmission season and geographic range, where there may be a potential for transmission cycles to occur [83, 84]. These models, which focus on transmission *potential,* can, however, over-predict both the impact of climate and current disease distributions. More conservative projections based on statistical approaches, such as that by Rogers and Randolph [85] have suggested negligible change in global malaria distributions. These are based on *current* global distributions and statistical estimates of existing incidence risk. It is difficult to explicitly incorporate nonclimatic factors such as health care, local habitat, and vector control into global malaria models; these determinants are therefore generally absent from global projections, though efforts have been made to integrate and reflect socioeconomic vulnerability and adaptive capacity into global scenarios in a general sense [9]. Rogers and Randolph, for example, acknowledge this challenge conceding that the model predictions are less reliable in marginal areas, where mosquito life-spans barely exceed incubation periods for the parasite, and acknowledging that nonclimatic factors are particularly important in determining the balance of transmission in these areas. Van Lieshout et al. [9] note that more accurate integration of socioeconomic variables in malaria modeling will require research at regional and national scales.

Research has been conducted on the potential for climate impacts on malaria in northern countries, particularly, the UK. Kuhn et al. [86], in an analysis of the risk of malaria re-emergence in Britain, concluded that despite an increase in the transmission potential due to climate change, the importance of nonclimatic factors (including medical systems, socioeconomic conditions, and agricultural changes) are likely to prevent emergence of endemicity. This example raises a second important point: the focus of climate-malaria models and assessments on distributions and spread of *endemic* malaria. While this is certainly justified in the prioritization of global health priorities and infectious disease burden, it does not negate the potential for climate impacts on sporadic autochthonous or imported cases in marginal or peripheral regions. Given the importance of nonclimatic factors on malaria transmission in marginal regions such as Canada, and using an assumption that nonendemic malaria incidence is of research and public health relevance, global climate-malaria models cannot be used to infer risk in such regions. That is to say that while existing models are rigorously developed and valuable to global climate-malaria projections, these models are not designed to predict changing risk in nonendemic areas, where climatic conditions for transmission are marginal.

5. Climate Change and Malaria in Canada

In the case of Canada and other developed regions on the periphery of the climatic range of malaria transmission, nonclimatic and local factors are important determinants affecting the balance of transmission potential. For these regions, with our current information, it is difficult to quantify whether increased climatic suitability would be sufficient to push the probability of malaria transmission beyond the threshold at which current localized and social factors become insufficient to inhibit transmission. Given Canada's northern climate and well-established social, health and economic systems, Canada is at negligible risk of experiencing endemic or regular malaria transmission despite the presence of competent *Anopheles* vectors [32, 87]. The questions of climate change impacts on malaria risk in Canada are not so much whether Canada will become an endemically infected country, but more whether changing climate determinants will have an impact on current incidence, and whether we can expect to see cases of locally-acquired autochthonous transmission in Canada. The answers to these questions require simultaneous evaluation of potential climatic effects

as well as trends in nonclimatic determinants of malaria transmission.

Predictions of shorter winters and increasing spring and summer temperatures, including prolonged summer heat waves [88, 89], could promote mosquito abundance and parasite replication in the summer in Quebec, Ontario, and British Columbia. Conversely, predictions of decreased summer rainfall, particularly in southern BC where there are already summer rainfall deficits [88, 89], could reduce mosquito survival. Southern Quebec and Ontario already have hot, humid summers with extended periods of high temperatures. Hotter summers, which are predicted by climate change in this region [88, 89], may result in a decrease in the number of consecutive warm days required for the parasite to develop within the mosquito and for the mosquito to become infectious. Additionally, milder winters and spring increases in precipitation in Ontario and Quebec [88, 89] may promote early mosquito abundance or increased winter survival of infective mosquitoes. These represent presumed potential impacts, though a number of descriptive reviews and qualitative assessments—particularly related to West Nile Virus—have suggested that climate changes will affect mosquito abundance and distributions in Canada [54, 87, 90–93], no quantitative models or results have yet been published to more certainly explore the potential impact of climate change on Canadian mosquito vectors.

In recent decades, there have been several years when Toronto has experienced more than 30 consecutive days above 18°C (Figure 3(a)), conditions potentially supporting *P. vivax* development in the vector. In 2002 and 2005, there were sufficient warm days to allow for two full replication cycles. The actual time required for parasite development depends upon an accumulation of "degree-days," a count of the cumulative number of days when temperatures exceed the minimum threshold for development, with each degree above the threshold contributing to an additional degree-day [1, 49, 82]. These days do not necessarily need to be consecutive; some parasite species can survive temperatures below and above the minimum threshold and continue development once temperatures rise again [35], although these relationships are poorly understood, particularly in northern latitudes.

Projected temperatures for Chatham in southwestern Ontario [47, 49] suggest a doubling of the summer period capable of supporting parasite development (based on a period of 30 days over 18°C) within the next 50–75 years (Figure 3(b)). Given that many of these days are well over 18°C and the number of days required for replication decreases at higher temperatures, these estimates can be considered conservative. This trend may be generalizable within the southern Ontario region, and while the degree of warming predicted varies among climate models and emissions scenarios, all model predictions in the IPCC FAR [2] and the Canadian National Impacts Assessment [94] indicate a warming trend in the Canadian regions where competent malaria vectors currently exist.

These projections can be placed within the context of global malaria modeling. Van Lieshout et al. [9], for example, suggest that regions where the transmission season increases from 0 to 1 or 2 months per year may experience large increases in population at risk. Canada fits within this range, and may experience increases of up to 3 months per year (Figure 3(b)). Van Lieshout et al. [9] also note that this transmission will be unstable (or sporadic) and that absolute risk remains low. Within the context of a national public health system such as Canada's, however, even low risk of emerging sporadic malaria is of importance for public health services and programming.

Changes in the variation and extremes of rainfall and temperature may be as important as trends in average temperature conditions for transmission potential. Southern Quebec is projected to experience both increases in average summer rainfall as well as more frequent and extreme rainfall events. Despite predictions of reduced summer rainfall in southern Ontario, summer rain is expected to occur as more frequent extreme rainfall events. Higher temperatures and drought conditions, followed by heavy rainfall, can provide ideal conditions for increasing mosquito abundance and reducing predator populations [87]. If combined with sufficiently extended periods of hot weather to support parasite development, these conditions could further increase transmission potential. Locally transmitted cases of malaria in Suffolk, New York, for example, occurred after heavy rainfall during a particularly hot and dry summer in 1999 [95]. This scenario is less likely in southern British Columbia, where increased rainfall is more likely to occur in the winter rather than the summer [88, 89].

Climate change could be expected, therefore, to increase the potential for locally transmitted, autochthonous malaria in Canada. Climate changes, however, are only one of several determinants of malaria transmission. Endemic malaria transmission occurred in Canada during the 1800s, for example, when temperatures were cooler than today [96]. It is, however, the *combination* of changes in multiple transmission determinants of *sporadic* autochthonous malaria that is of interest.

6. A Systems Approach to Malaria in Canada

Drawing on the guidelines outlined in Table 1, we can characterize and assess the role of indirect climate impact and nonclimatic determinants of transmission, as well as their interactions. Systems frameworks employ a variety of conceptual tools for system characterization. Here, we adapt the life cycle model of malaria to include the broader determinants of transmission (Figure 4). The basic reproductive number (R_0) of malaria is determined by the biting rates of mosquitoes, and by infection of mosquitoes and humans, which are constrained by the life cycle of mosquitoes and parasites [97]. R_0 represents the transmission potential of a disease, that is to say, the number of secondary cases expected to arise from a primary case in a naïve population [97]. Empirical malaria modeling is often restricted to quantification of these inner parameters and their proximate determinants. The magnitude of these transmission parameters, however, is influenced by a range of mediating variables (outer circle), many of which vary at regional/ local scales or are related to sociopolitical rather than biological systems. As

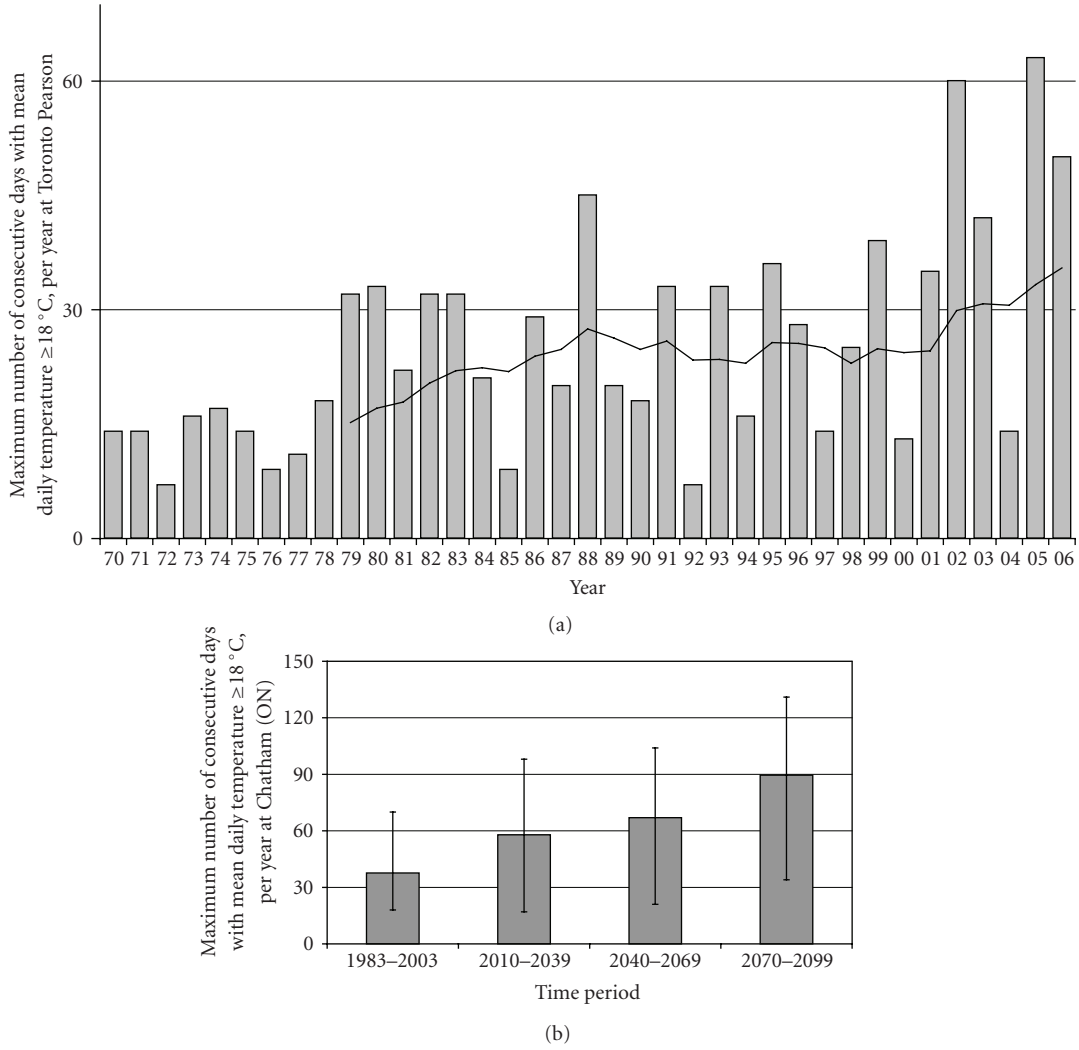

Figure 3: (a) Annual number of consecutive days ≥18°C, Toronto. Bars indicate the number of consecutive days per year that temperatures ≥ 18°C. A trendline (solid line) shows the 10-year moving average for the data. The trendline suggests that in the last few years, we have begun to experience sufficiently prolonged summer warm periods to support parasite replication and malaria transmission potential. In 2002 and 2005, the number of days above 18°C was sufficient to support 2 cycles of *P. vivax* replication. These data should be considered conservative since each degree-day ≥18°C will reduce the remaining time required for parasite replication. Additionally, breaks in consecutive warm days ≥18°C do not necessarily prohibit continued development once temperatures rise [35]. Source of climate data: Environment Canada [46]. (b) Annual number of consecutive days ≥18°C projected for 2010–2099, Chatham (ON). Bars indicate the number of consecutive days per year that temperatures are projected to reach or exceed 18°C. Error bars indicate the range of values during each time period. The climate change projections were obtained from interpolation (for Chatham, Ontario) of output from the CGCM2 (Canadian Coupled Global Climate Model 2) [47] that were downscaled using LARS-WG stochastic weather generator. LARS-WG was calibrated with 30 years of daily weather observations at Chatham (and its predecessors) obtained from the Environment Canada database. The output used here was obtained using emissions scenario A2 (business as usual). The data and methodology used here are the same as described in Ogden et al. [49]. The projected trend shown here indicates increasingly extended summer warm periods sufficient to support multiple parasite replication cycles.

we illustrate, climatic determinants represent only one set of variables affecting malaria transmission parameters.

Of the variables shown in Figure 4, incidence and parasitemia in the mosquito population are perhaps the most likely to experience temporal variation in Canada. Given the low incidence of malaria and current absence of local transmission in Canada, variations in vector control, personal protection measures, housing, socioeconomic variables, differential immunity, and local habitat are unlikely to notably influence transmission. Delays in diagnosis and treatment of malaria in Canada are already a concern [59, 98] and would prolong duration of infection as well as increase transmission potential under conditions of local transmission.

Climate change may have indirect effects on Canadian malaria incidence by affecting the nonclimatic determinants of transmission. For example, any increased incidence of malaria in countries to or from which Canadians travel would affect the magnitude of imported cases and the risk

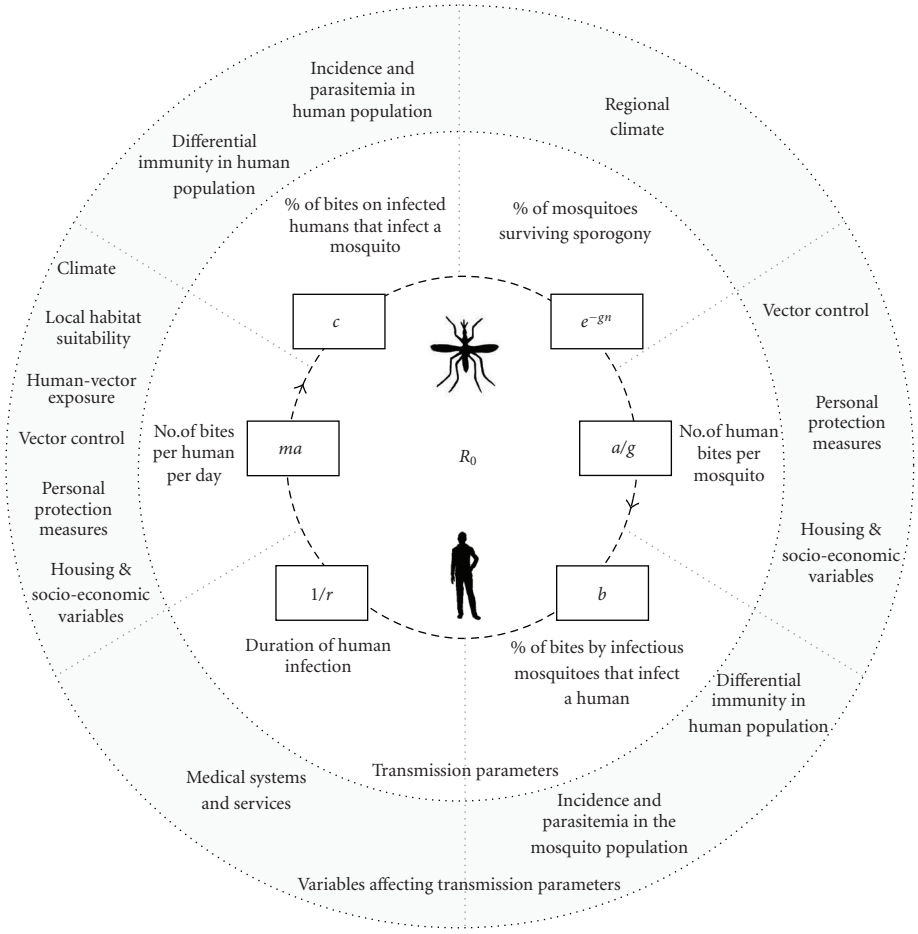

Figure 4: Malaria life cycle model. The inner model of malaria transmission parameters is based on a diagram and parameters from Smith et al. 2007 [97]. Parameter definitions: R_0 (basic reproductive number) = $ma^2\,bce^{-gn}/rg$, a (human feeding rate): the number of bites on a human, per mosquito, per day, b (transmission efficiency): the probability that a human becomes infected from a bite by an infectious mosquito, c (transmission efficiency): the probability that a mosquito becomes infected from a bite on an infected human, g (death rate of mosquitoes): expected lifespan of a mosquito in days: 1/g, m: ratio of mosquitoes to humans, n (incubation period): number of days required for the parasite to develop within the mosquito, $1/r$: duration of infection in humans.

of introduction of parasites into Canadian mosquito populations. This would affect incidence and parasitemia levels in the human population, as well as transmission efficiency (parameters b and c in Figure 4). Indirect climate change impacts on health vulnerability, socioeconomic status, reduced resources available for vector control measures or health systems, as well as trends in population growth, other diseases, overall poverty and health, and drug use or resistance [80–82, 99], though difficult to quantify, are potentially significant. Similarly, changes in climate such as warmer and longer summers could result in behavioural shifts—such as increased or extended seasonal use of parks and backyard BBQs—that could indirectly affect human-vector exposure and biting rates. Air-conditioning use in southern Canada, which can be expected to increase with warmer summer temperatures, may provide a protective effect, reducing transmission by limiting human exposure to vectors [100].

Increasing immigration and increasing international travel [58, 60, 101, 102]—particularly Canadian immigrants returning to visit friends and relatives in malaria-endemic countries—may increase the likelihood of imported cases, as well as the potential for introduction of parasites into the Canadian mosquito population. The proportion of Canadian immigrants originating from malarial areas has increased significantly in the past 50 years [103] and more Canadians are traveling more often to malarial destinations in Africa, Asia, and South America [104]. Resulting increases in infected and infectious individuals in Canada may increase the potential for transmission of parasites to the local mosquito population as well as potential occurrence of transfusion-transmitted malaria [105]. Canada has, in fact, already recorded malaria cases that may be associated with climate, immigration, and international travel. A dramatic increase in imported *P. vivax* cases in 1995–97 was likely attributable to Canadians of Indian origin who visited the Punjab region of India during a *P. vivax* outbreak associated with higher than normal temperatures and precipitation during a strong El Niño year [59, 64, 65, 106–108]. This

Table 3: Trends in the determinants of malaria incidence and transmission in Canada.

Driving factors	Potential effect on determinants of malaria transmission	Impact on malaria risk in Canada	Impact on malaria transmission parameters (Figure 4)
Climate change	Improved mosquito habitat in Canada, increased vector populations	Increased probability of local transmission	$\uparrow ma$ $\downarrow g\ (\uparrow e^{-gn})$
	Extension of the annual period available for parasite replication in mosquitoes in Canada	Increased probability of local transmission	$\uparrow ma$ $\downarrow g\ (\uparrow e^{-gn})$
	Changes in the distribution and/or incidence in countries outside of Canada to/from which Canadians travel	Impact on the number of imported cases Probability of local transmission uncertain	$\uparrow$ or $\downarrow b$ $\uparrow$ or $\downarrow c$
Increasing immigration	Increased introduction of infected individuals	Increased number of imported cases Increased probability of local transmission	$\uparrow b$ $\uparrow c$
Increasing international travel	Increased introduction of infected individuals	Increased number of imported cases Increased probability of local transmission	$\uparrow b$ $\uparrow c$
Drug resistance	Decreased efficacy of prophylaxis	Increased incidence and mortality	$\uparrow 1/r$
Delayed diagnosis	Increased gametocyte incidence	Increased probability of local transmission	$\uparrow 1/r$

example demonstrates the potential for climate impact outside Canada to affect malaria incidence in Canada. Increased incidence and parasitemia in the human population is likely to be most pronounced in urban areas, where travel transit and immigration are the highest.

The on-going, and sometimes rapid, emergence of parasite resistance to antimalarials also has the potential to affect Canadian malaria incidence. Drug resistance has resulted in the emergence and re-emergence of malaria around the world, and it considered to be one of the greatest challenges to global malaria control today [109]. Antimalarial resistance can increase the incidence, severity, and cost of travel-related malaria in Canada by decreasing the efficacy of traveler prophylaxis, complicating selection of prophylaxis and treatment regimes, and increasing the potential for treatment failure. A summary of key trends in the determinants of malaria incidence in Canada is provided in Table 3.

7. Conclusion

Our characterization and assessment of the changing climatic and nonclimatic determinants of malaria indicates that Canada may experience increasing imported incidence as well as the potential for emergence of sporadic cases of autochthonous malaria. Our analysis provides a *qualitative* and exploratory assessment of potential climate impacts within the context of other Canadian disease determinants and trends. Whether these trends and parameters would be *quantitatively* sufficient to tip the balance towards sporadic autochthonous transmission is unknown and requires further study.

While well within the capacity of Canada's health system to address, the potential for changes in malaria incidence would require targeted and strategic shifts in health service programming, physician/technologist education and training, travel agent education and travel clinic referral, education of the public, and surveillance. Targeted research to quantify the sensitivity of potential local mosquito transmission to key parameter changes would require the development of process-based models or equivalent empirical models to simulate sporadic incidence. This is consistent with global research recommendations identifying the need for further research on malaria modeling at the regional or national scales and integrating regional environmental and socioeconomic variation [9].

While not quantitatively sufficient to project incidence increase, the results of this review and characterization qualitatively support the merit of targeted regional research to quantify projected transmission potential in Canada. Such an endeavour would be of importance to public health in Canada, but is also of relevance to broader questions related to the impact of climate change on infectious disease occurrence. This review, while regional, highlights the utility of systems frameworks in characterizing potential health risks not readily identified or addressed by global models or climate attribution modeling.

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