Airborne dust and high temperatures are risk factors for invasive bacterial disease

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Background: The Sahel region of West Africa has the highest bacterial meningitis attack and case fatality rate in the world. The effect of climatic factors on patterns of invasive respiratory bacterial disease is not well documented.

Objective: We aimed to assess the link between climatic factors and occurrence of invasive respiratory bacterial disease in a Sahel region of Niger.

Methods: We conducted daily disease surveillance and climatic monitoring over an 8-year period between January 1, 2003, and December 31, 2010, in Niamey, Niger, to determine risk factors for bacterial meningitis and invasive bacterial disease. We investigated the mechanistic effects of these factors on *Streptococcus pneumoniae* infection in mice.

Results: High temperatures and low visibility (resulting from high concentrations of airborne dust) were identified as significant risk factors for bacterial meningitis. Dust inhalation or exposure to high temperatures promoted progression of stable asymptomatic pneumococcal nasopharyngeal carriage to pneumonia and invasive disease. Dust exposure significantly reduced phagocyte-mediated bacterial killing, and exposure to high temperatures increased release of the key pneumococcal toxin pneumolysin through increased bacterial autolysis.

Conclusion: Our findings show that climatic factors can have a substantial influence on infectious disease patterns, altering density of pneumococcal nasopharyngeal carriage, reducing phagocytic killing, and resulting in increased inflammation and tissue damage and consequent invasiveness. Climatic surveillance should be used to forecast invasive bacterial disease epidemics, and simple control measures to reduce particulate inhalation might reduce the incidence of invasive bacterial disease in regions of the world exposed to high temperatures and increased airborne dust. (J Allergy Clin Immunol 2017;139:977-86.)

Key words: *Meningitis, climate,* Neisseria meningitidis, Streptococcus pneumoniae, *pollution, dust*

The 1000-km-wide semiarid Sahel region, which lies between the Sahara desert to the north and the Sudanese Savanna to the south, has the highest attack rate (10 per 100,000) and case fatality rates (15%) in the world for bacterial meningitis.[1,2] This region, which is also known as the meningitis belt, comprises 350 million persons at risk across 21 countries.

Niger, a Sahel country, has a long history of meningitis epidemics, with recent large-scale outbreaks occurring in 2000, 2003, and 2009. *Neisseria meningitidis* serogroups A and X and *Streptococcus pneumoniae* are the main causative agents.[3,4] Meningitis outbreaks in Niger show strong seasonality, suggesting climatic factors could play a role in disease mechanisms,[5-10] but these studies focus on all-cause meningitis, and little is known about the specific effect of climate on bacterial meningitis.

The dry and dusty Harmattan winds that blow between November and May are a unique defining feature of the West African climate and have been associated with outbreaks of meningitis.[11] On its passage over the desert, the Harmattan wind picks up fine fractions of Saharan dust particles (mostly particulate matter <10 μm).[11] The sheer amount of dust in the air can severely limit visibility and sometimes block the sun for several days, which is comparable with a heavy fog. Indeed, the inverse correlation between visibility and particulate matter concentration has been demonstrated in Niger and elsewhere.[12,13] Dust is thought to have a negative effect on health, increasing morbidity caused by diseases of the upper and lower respiratory tract.[14]

A recent study using a global atmospheric chemistry model has suggested that outdoor air pollution leads to 3.3 million premature deaths per year worldwide, with natural sources of particulate material (predominantly desert dust) responsible for 600,000 (18%) of those deaths.[15] In large parts of North and East Africa, the Middle East, Central Australia, and Central Asia, natural sources of small particulate material, such as desert dust, make a larger contribution to mortality than more recognized pollution sources, such as industry, traffic, energy, and agriculture. Thus

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Abbreviations used
A600: Absorbance at 600 nm
CFU: Colony-forming units
OPKA: Opsonophagocytic killing assay
PLY: Pneumolysin

understanding the link between desert dust inhalation and mortality and the climatic factors that influence levels of airborne dust is key to disease control in affected areas.

Long-term forecasting and identification of climatic risk factors would help public health decision makers improve early warning systems and would help the scientific community to identify physiologic factors implicated in the development of invasive diseases. Statistical forecasting models that integrate climatic factors, linking environmental and epidemiologic surveillance, could act as early warning systems of infectious disease epidemics. Here we present findings from a study quantifying, on a daily scale, this link between climate and meningitis in Niamey, Niger. Furthermore, we model these effects *in vivo* by using experimental infection of mice.

METHODS

Ethics statement

Biological surveillance was performed by the national reference center of the Public Health Ministry of Niger, CERMES (Centre de Recherche Médicale et Sanitaire), which is part of the meningitis national control program.

Study area and meteorology

The study area was defined as a radius of 50 km around the meteorological station of the international airport of Niamey, Niger, and constituted a homogeneous geographic area for which climatic factors were measured daily. These measures comprise minimal and maximal temperatures, minimal and maximal relative humidity, mean wind speed, mean visibility (defined by the World Meteorology Organization as the maximal distance from which an observer can distinctly see an object on a horizontal plane), and rainfall. Seasons were defined by the National Forecasting Direction (Direction de la Météorologie Nationale) of Niger.

The population of the study area was 1,099,057 for the median year 2006. Cases of meningitis are registered daily, and all cases within the study area confirmed by means of culture, PCR, or both were enrolled between January 1, 2003, and December 31, 2010. Thirty-four health care facilities were involved. Full details can be found in the Methods section in this article's Online Repository at www.jacionline.org.

Mouse model of *S pneumoniae* infection

All animal experiments were performed at the University of Liverpool in accordance with the Animal Scientific Procedures Act 1986 and with the prior approval of the UK Home Office (PPL 40/3602) and the University of Liverpool ethics committee.

Sex- and age-matched MF1 mice (Charles River, Margate, United Kingdom) were used. Asymptomatic nasopharyngeal carriage was established in mice by means of intranasal infection, as described previously.[16,17] For particle inhalation experiments, 2 days after infection, mice underwent intranasal administration of 50 mg/mL silicon dioxide (dust; mean particle size, 10 μm; Sigma, Dorset, United Kingdom) or PBS as a control. This was repeated at 4 days after infection, and mice were culled at 7 days after infection or if invasive disease signs (as described by the scheme of Morton and Griffiths[18]) progressed to visible lethargy. For heat exposure experiments, mice were put in a heat box at 40°C for 10 minutes before and for 20 minutes after induction of nasopharyngeal carriage. Control mice were housed at 21°C throughout. The nasopharynx, lungs, brain, and blood were removed and homogenized in PBS before plating on blood agar for assessment of tissue colony-forming units (CFU). Full details can be found in the Methods section in this article's Online Repository.

Pneumolysin detection ELISA

Sandwich ELISA was performed with mouse anti-pneumolysin (PLY; PLY-4; Abcam, Cambridge, United Kingdom) and rabbit anti-PLY antibody (Abcam). Absorbance at 405 nm was read with a Multiskan Spectrum microplate reader (Thermo Scientific, Waltham, Mass). Full details can be found in the Methods section in this article's Online Repository.

Opsonophagocytic killing assay

Opsonophagocytic killing assays (OPKAs) were performed, as previously described,[19] with minor modifications. Briefly, J774 mouse macrophages or HL-60 human neutrophils were incubated with 50 μg/mL silicon dioxide for 1 hour of shaking (175 rpm) before addition of opsonized *S pneumoniae* and complement. CFU values were determined after a further 45 (HL-60) or 60 (J774) minutes of incubation. Full details can be found in the Methods section in this article's Online Repository.

Measurement of autolytic activity

Triton X-100–induced autolysis assays were performed, as described by Houston.[20] Full details can be found in the Methods section in this article's Online Repository.

Hemolytic assay

Overnight cultures of *S pneumoniae* serotype 2 (strain D39) and its isogenic autolysin (LytA)–deficient mutant were subcultured in brain-heart infusion media and incubated at 37°C or 40°C to an absorbance at 600 nm (A600) value of 1.0. Cells were then pelleted, and the supernatant was removed and filter sterilized. Hemolytic activity against sheep red blood cells was measured, as described previously.[21]

Statistical analysis

A descriptive analysis was performed for the median and interquartile range of the climatic factors, with the range and coefficient of variation according to season. A Mantel-Haenszel χ^2 test was used to adjust the relative risk for a maximal temperature threshold of greater than 39.5°C on seasons with the statcalc program of Epi Info 6.04 software (Centers for Disease Control and Prevention, Atlanta, Ga).

A generalized additive model with a negative binomial family was used to regress a time series of daily counts of confirmed cases of meningitis with daily changes in climatic factors. Full details can be found in the Methods section in this article's Online Repository. All analyses were performed with R software (R Development Core Team, 2010, version 2.12.0).

Mouse model data were analyzed with GraphPad Prism software (GraphPad Software, La Jolla, Calif) by using ANOVA or a log-rank test with appropriate posttesting. Results with P values of less than .05 were considered significant. Data represent means ± SEMs, unless otherwise indicated. Data were assessed for normality by using the D'Agostino-Pearson (omnibus K2) test.

RESULTS

We conducted daily disease surveillance and climatic monitoring over an 8-year period between January 1, 2003, and December 31, 2010, in Niamey, Niger. Over the 8 years, 893 confirmed cases of bacterial meningitis were recorded within the study site. Epidemics ranged in size from 36 in 2008 to 305 in 2006, with corresponding attack rates of 3.3 to 27.8 10^{-5} (Table I).