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Non-linear effect of temperature variation on childhood rotavirus infection: A time series study from Kathmandu, Nepal

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Abstract

Introduction: This study aimed to investigate the effects of temperature variability on rotavirus infections among children under 5 years of age in Kathmandu, Nepal. Findings may inform infection control planning, especially in relation to the role of environmental factors in the transmission of rotavirus infection.

Methods: Generalized linear Poisson regression equations with distributed lag non-linear model were fitted to estimate the effect of temperature (maximum, mean and minimum) variation on weekly counts of rotavirus infections among children under 5 years of age living in Kathmandu, Nepal, over the study period (2013 to 2016). Seasonality and long-term effects were adjusted in the model using Fourier terms up to the seventh harmonic and a time function, respectively. We further adjusted the model for the confounding effects of rainfall and relative humidity.

Results: During the study period, a total of 733 cases of rotavirus infection were recorded, with a mean of 3 cases per week. We detected an inverse non-linear association between rotavirus infection and average weekly mean temperature, with increased risk (RR: 1.52; 95% CI: 1.08-2.15) at the lower quantile (10thpercentile) and decreased risk (RR: 0.64; 95% CI: 0.43-0.95) at the higher quantile (75th percentile). Similarly, we detected an increased risk [(RR: 1.93; 95%CI: 1.40-2.65) and (RR: 1.42; 95%CI: 1.04-1.95)] of rotavirus infection for both maximum and minimum temperature at their lower quantile (10th percentile). We estimated that 344 (47.01%) cases of rotavirus diarrhoea among the children under 5 years of age were attributable to minimum temperature. The significant effect of temperature on rotavirus infection was not observed beyond lag zero week.

Conclusion: An inverse non-linear association was estimated between rotavirus incidence and all three indices of temperature, indicating a higher risk of infection during the cooler times of the year, and suggesting that transmission of rotavirus in Kathmandu, Nepal may be influenced by temperature.

Key words: Rotavirus, Environmental factors, Epidemiology, Children, Nepal

Introduction

Rotavirus infection was globally responsible for 29.3% (146,500 deaths) of all diarrhoea-related deaths among children below 5 years of age in 2015 (1), and 128,500 deaths among the same population in the following year (2). In the context of Nepal, rotavirus infection has been estimated to cause 50-100 deaths per 100,000 infection cases reported each year among children under 5 years of age (3). The worldwide distribution of rotavirus infection, regardless of the hygiene levels, socio-economic status, and food and air quality, highlights the important role of weather factors in the transmission of rotavirus diarrhoea (4-6). Rotavirus infection has been reported to show distinct seasonality with a winter peak in temperate regions, while a less pronounced seasonality has been observed in tropical regions where the infection prevails all year round (7) . In addition to tropical countries, year-round prevalence of rotavirus infection has been reported in several South Asian countries, including Bangladesh (7-10).

Kathmandu city lies in a warm, temperate zone, situated at an average elevation of 1,400 metres above sea level, and is characterised by a subtropical humid to subtropical highland climate. The annual average summer temperature varies from 28 °C to 30 °C, with the average winter temperature varying between 8 °C and 10 °C. The city receives monsoon rainfall with an average of 1,407 mm of rain per annum. According to the census of 2011, the total population of Kathmandu district was 1,744, 240 with a population density of 4,416 per sq. km. The population of the under five year age group was 111, 600 (11). With a low Gross National income of around US$2,472 and a lower Human Development Index (0.578), the socioeconomic development status of people living in this region is below the average value for South Asia countries (12). It is estimated that nearly 350 MLD (million litre per day) of waste water is produced in Nepal, of which only 5% is being treated currently (13). Direct discharge of untreated waste water and sewage into the water bodies is a common practise in

Kathmandu that has led to transformation of water bodies into open sewage with contaminant and entero pathogens. In context of Kathmandu, a waste water treatment plant of capacity 17.3 MLD is partially in operation to clean up a major water body i.e., Bagmati river (13).

A hospital-based program of rotavirus infection surveillance was initiated in 2005 at Kanti Children Hospital, Kathmandu, through a collaboration between the Institute of Medicine, Tribhuvan University, Nagasaki University, and the University of Liverpool (11). This program was later integrated into the Asian Rotavirus Surveillance Network to continue rotavirus monitoring in Kathmandu, Nepal (12). Over the last decade, the annual prevalence of rotavirus infection among children under 5 years in Kathmandu has been reported to be between 22.2% and 33%. Infections occur year-round, with a higher incidence during the winter months (14-17). A number of studies have used rigorous statistical methods to estimate the effects of climatic factors on rotavirus infections, while controlling for confounding variables and seasonal and long-term variation (6, 18-23). However, few studies have been carried out in a warm, temperate region like Kathmandu city.

Given the lack of a unifying explanation for varying degrees of rotavirus seasonality across the world (24), investigating the role of local climatic conditions may provide insights into the transmission and epidemiology of rotavirus infection for a given location (4), especially for the city of Kathmandu with its unique climatic and socioeconomic characteristics. Although rotavirus burden has been consistently substantial over the past few decades, a rotavirus vaccine has not been introduced into the National Immunization Program of Nepal (17). As such, better understanding of the role of climatic factors and seasonal patterns in the spread of rotavirus in Kathmandu may support the implementation of effective vaccine intervention programs in the future (18). Discerning the role of environmental factors in virus transmission may help public health authorities design a more systematic plan for the prevention of rotavirus infection in Kathmandu during high risk periods. Furthermore, it may facilitate exploration of the broader effects of climate variation and climate change on rotavirus transmission in the Nepalese context (5). Thus, this study aims to explore the association between climatic factors and rotavirus infections among children younger than 5 years in the warm temperate zone of Kathmandu, Nepal, and to provide scientific evidence to local health authorities to support the design of an appropriate intervention program for the prevention of rotavirus infections in the context of a changing climate.

Methods

Data source

We included the weekly reported counts of laboratory-confirmed rotavirus cases among children under 5 years of age attending Kanti Children Hospital, Kathmandu, based on the illness onset date across a four year period (2013 – 2016) as the primary outcome. Surveillance data on the weekly count of laboratory-confirmed rotavirus cases were obtained from the Public Health Research Laboratory, Institute of Medicine, Tribhuvan University, Nepal (17), a member site of the Asian Rotavirus Surveillance Network (25). All participants enrolled in the surveillance program were screened for the presence of rotavirus antigen in their stool samples using Enzyme-linked immune sorbent assay (ELISA) kits (ProSpecT^{TM}Rotavirus, Oxoid Ltd, UK), followed by confirmation via reverse-transcription polymerase chain reaction (RT-PCR) (17).

Daily climate data from Kathmandu district were obtained from the Department of Hydrology and Meteorology, Nepal, and weekly means were calculated for temperature variables and relative humidity. Weekly cumulative rainfall totals were calculated from daily records. Meteorological data from Kathmandu Airport station were used as these provided better coverage of the study location and more complete data.

Statistical analysis

Spearman’s correlation analysis was carried out to assess the association between weekly rotavirus infections and climatic variables including temperature (maximum, mean and minimum), mean relative humidity and rainfall over the study period (*Supplementary figure S1*). We conducted a time series regression analysis to examine the relationship between the weekly average of climate variables and rotavirus infection count using a generalised Poisson regression model. We first checked the distribution of the weekly rotavirus count for over-dispersion using a regression-based test for the Poisson regression model (26) to ensure the data were not over-dispersed (*Supplementary figure S2*). We then carried out the univariate regression analysis for all climatic variables against the outcome variable i.e., total weekly count of rotavirus infection among children (<5 years). Three different indices of temperature (mean, maximum, and minimum) were the only variables significantly associated with rotavirus infection in the univariate models. Hence, to avoid multicollinearity, we finally defined three separate multivariable regression models for each temperature index by adjusting the possible confounding effects of relative humidity and rainfall. Seasonality and long-term effects were also adjusted in the model using Fourier terms up to the seventh harmonic and a function of time, respectively. We used distributed lag non-linear models using the DLNM package in software R (27) to model the non-linear and possible lagged effects of temperature on rotavirus infection. The DLNM model facilitates simultaneous exploration of the exposure-lag-response relationship between the predictor and outcome along a two-dimensional array of exposure and lagged effect (27). Using the DLNM package we modelled the effect of temperature with natural cubic splines of 3 degrees of freedom. Guided by previous research on the effect of climate variation on rotavirus infection (6, 18, 19), we set a lag period of 4 weeks to model the delayed effect using natural cubic splines of 3 degrees of freedom. The selection of spline type for both exposure and lag function was based upon the Akaike Information Criterion (AIC). The confounding effects of both rainfall and humidity were adjusted using the natural cubic spline of 3 degrees of freedom. Risk ratios of rotavirus infection in response to temperature variation were estimated with reference to the weekly median temperature value. In summary, the final model took the following form:

Log [E(y)] = α + (cbo_temperature) + time (Fourier, 7 harmonics/year) + NS (mean_rh, df=3) + NS (rainfall, df=3) + f (time)

where, E(y) is the expected weekly case count, cbo temperature is the cross basis matrix for three different indices of temperature, Fourier represents the Fourier (trigonometric) terms, NS (mean_rh) and NS (rainfall) are the weekly average humidity level and weekly cumulative rainfall total with natural cubic splines of 3 degrees of freedom, and time is the linear function of time.

The sensitivity of the model was analysed by changing the number of Fourier harmonics from 3 to 9 harmonics per year. The model with lowest AIC value and low (partial) autocorrelation was selected as the robust model (*Supplementary table S1 and figure S3).*

Attributable risk estimation for rotavirus infection cases

We calculated the attributable risk of rotavirus infection against temperature exposure (maximum, mean and minimum) from risk estimates of a distributed lag non-linear model, using "attrdl.R" function in software package R, as previously described (28). Briefly, the backward attributable fraction and attributable number were calculated using risk estimates obtained from the dlnm model with reference to a counterfactual scenario of minimum exposure, indicated by the lowest relative risk, for maximum temperature, mean temperature and minimum temperature respectively.

Results

During the study period (2013-2016), a total of 733 cases of rotavirus infection were recorded among children under 5 years of age attending Kanti Children's Hospital, Kathmandu, Nepal. The average weekly number of cases of rotavirus infection among children younger than 5 years reported during the study period was 3 (Table 1).

The overall distribution pattern of rotavirus infection cases during the four year period showed year-round prevalence with a minimum of one case and a maximum of 11 cases reported during the total 212 weeks included in study (Figure 1). Age-specific distribution patterns of the cases and different variables recorded during the study period have been published elsewhere (17).

Relationship with maximum temperature

We estimated an inverse nonlinear association between rotavirus infection among children (<5 years) and maximum temperature, with an increased risk (RR: 1.93; 95% CI: 1.40-2.65) at the lower quantile (10th percentile) and decreased risk (RR: 0.95; 95% CI: 0.84-1.11) at the higher quantile (75th percentile) (Figure 2 & 3). The risk of rotavirus infection appears to increase at the 99th percentile of the temperature data, but the effect was not significant (Figure 2). The effect of maximum temperature on rotavirus was not significant beyond the lag period of zero weeks (*Supplementary figure S4*).

Relationship with mean temperature

As per the effect of maximum temperature, we also observed an inverse nonlinear relationship between mean temperature and rotavirus infections, with an increased risk (RR: 1.52; 95% CI: 1.08-2.15) at the lower quantile (10th percentile) and decreased risk (RR: 0.64; 95% CI: 0.43-0.95) at the higher quantile (75th percentile) (Figure 3). Akin to the effect of maximum temperature, the effect for mean temperature did not persist beyond the lag period of zero weeks *(Supplement figure S4).*

Relationship with minimum temperature

There was an inverse relationship between minimum temperature and rotavirus infection among children (<5 years), with increased risk (RR: 1.42; 95% CI: 1.04-1.95) at the lower quantile (10th percentile) and decreased risk (RR: 0.60; 95% CI: 0.46-0.78) at the higher quantile (75th percentile). Unlike maximum temperature, the protective effect of increased temperature against rotavirus infection continued beyond the 99th percentile (Figure 2). Similarly, the effect of minimum temperature on rotavirus infection was also not observed beyond the lag period of zero week (*Supplementary figure S4*).

Overall, an inverse nonlinear association was detected between all three indices of temperature and rotavirus infection, with an increased risk of infection at the lower quantile and decreased risk at the higher quantiles (Figure 2). Relative humidity and rainfall were not found to be associated with rotavirus infection among children (<5 years).

Rotavirus infection risk attributable to temperature

The estimates of total backward attributable risk (attributable number and attributable fraction) of rotavirus infection specific to different temperature indices are reported in Table 2. As outlined in the table, 47% (eCI: 24-60) of cases of rotavirus diarrhoea among the children under 5 years of age were attributable to minimum temperature.

Discussion

We have used the state of art modelling technique (distributed lag nonlinear model), widely used in environmental epidemiology, to assess the short-term effect of temperature variability on the risk of rotavirus infection in Kathmandu, Nepal, while adjusting for seasonality, long-term variation, lag-effect and other possible meteorological factors such as rainfall and humidity. Despite the year-round detection of rotavirus infection, our study has found that lower temperature poses a higher risk of rotavirus diarrhoea incidence among children under 5 years of age in Kathmandu, demonstrating an inverse non-linear relationship with all three indices of temperature. Our findings with regard to the increased risk of rotavirus transmission on colder days of the year in Kathmandu supports public health practitioners and health policy-makers to design and deploy effective intervention programs for the prevention of rotavirus infection in Nepal, considering the climatic aspect of virus transmission. The Epidemiology and Disease Control Division of Nepal, in coordination with electronic and print media, might usefully broadcast special alerts to promote precautionary measures during winter high-risk periods, such as improved hygiene practices (e.g., hand washing, proper dispensing of baby diapers), and advice to reduce unnecessary physical interaction with children to prevent rotavirus transmission form carrier to susceptible children (29). In the absence of mandatory rotavirus vaccinations in Nepal, these findings further highlight the importance of identifying specific pathways (interactions between host and environmental factors) through which a higher risk of rotavirus transmission is mediated during colder days in Kathmandu.

Apart from the increased survival duration of viral particles at low temperatures, the biological reasons behind the higher transmission rate of rotavirus infection at lower temperature remains unclear (24). A plausible explanation for the increased risk of rotavirus infection at lower temperatures could be a due to poorer hygienic practices in cooler conditions, for example, reduced hand washing frequency during cold weather, which may increase chances of faeco-oral transmission of rotavirus (30). We speculate that the reluctance of people to leave a warm place, or the irregular availability of warm water in high-risk areas of sub-Saharan Africa and South Asia, may compromise the hand hygiene practices of mothers and caregivers during cold weather. In the context of Kathmandu district, 58.7% of households live in rental housing (31), and the average number of rooms per household in Nepal is 4.4 (32). Given the higher population density (4,416 person per sq.km) of Kathmandu district (11), and the lower number of rooms per household, it is highly likely that residents living in rented houses are living in overcrowded conditions. Indeed, an increased risk of rotavirus transmission in cold weather due to the overcrowding of people in rented households has been previously reported in England and Wales (6, 33). Hence, increased proximity among people living in overcrowded conditions is likely to cause more frequent physical interaction between individuals, especially between susceptible children and their caregivers, which may be a key facilitator of the transmission of rotavirus in Kathmandu city during cold weather. Similarly, the increased physical interaction between children and their caregivers during cold weather, facilitating airborne droplet-mediated transmission of rotavirus from asymptomatic adults to children, could be another plausible cause of virus transmission during these conditions (30). Meanwhile, some research has indicated that the higher transmission of rotavirus in colder conditions may not be directly related to lower temperatures *per se,* but rather, be the result of unfavourable conditions for rotavirus transmission during hot summer days, given the lower survival rate of rotavirus at higher temperatures and in higher humidity (4, 34).

The inverse association between rotavirus infection and temperature observed in our study is consistent with previous studies conducted in the Netherlands and Great Britain (6, 21), Spain(20), Australia (18), Turkey (35), Hong Kong (22) and Indonesia (36). These studies were conducted in a diverse climatic zones, ranging from cool temperate, dry temperate, sub-tropical coastal to a humid tropical, and spanned diverse socio-economic contexts, yet have reported similar findings to our results recorded in a sub-tropical highland climate, strengthening evidence for the negative association between rotavirus infection and temperature irrespective of the climate type. A multisite study including Bhaktapur city, Nepal, also reported an inverse association between rotavirus infection and temperature (23). Bhaktapur city shares its boundary with Kathmandu city, and both cities have similar climatic characteristics. Colston et al, (23), however, have adjusted for additional variables including soil moisture, solar radiation and specific humidity in their final model while estimating the effect of daily mean temperature on rotavirus infection in Bhaktapur city.

Two separate systematic reviews and meta-analyses on the seasonality of rotavirus in South Asian and Tropical countries have reported a 1.3% and 10% (95% CI: 6-13%) decrease in rotavirus infection respectively, with a 1°C increase in mean temperature (4, 37). These studies have only used mean temperature as the primary exposure variable, but we have used all three temperature indices to measure the exposure-response relationship between temperature and rotavirus incidence in our study. In addition, most of these studies have assumed a linear relationship between rotavirus and temperature except for one study in the Netherlands (4, 21). In contrast, we have used a distributed lag non-linear model to estimate non-linear and possible lag association between temperature exposure and risk of rotavirus infection, as the use of conventional modelling strategy using splines and an unconstrained distributed lag model to account for non-linearity may result in imprecise estimates with wider confidence intervals due to a higher correlation between lag terms used in the model (38).

Contrary to the findings of a majority of previous studies, a study from Bangladesh reported that a higher temperature (mean temperature) above a threshold (29 °C) was positively associated with an increased risk of rotavirus infection (19). For this reason, most of the high-temperature weeks coincided with heavy rainfall in the study site (Dhaka), a complex interaction of several climatic factors which might have driven the temperature-dependent effect modification on rotavirus infection in this study (6). Intriguingly, in our study, the risk of rotavirus infection appeared to increase with the increase in maximum temperature towards the 99th percentile; however, the result was not statistically significant and showed a very wide confidence interval, which could have stemmed from the relatively smaller sample size of rotavirus positive cases. Consequently, we checked for the weeks with a maximum temperature above 31 °C in our dataset, and discerned that mean relative humidity during these weeks was below the first quantile and that these weeks were dry with low rainfall (10 weeks out of the 14 weeks had cumulative rainfall <2.5 mm), suggesting that dry weather conditions with low humidity could have been responsible for the observed pattern of increased risk towards the higher percentile of maximum temperature. Several epidemiological and laboratory based studies have reported that low relative humidity and dry conditions can be conducive for insitu survival and higher transmission rate of rotavirus (4, 34, 37), which may explain the aberrant observation of an increased slope of exposure risk curve for maximum temperature, towards the 99th percentile in our study.

Besides the use of a distributed lag non-linear model, another strength of our study is the inclusion of minimum temperature as an exposure variable. Given the observed negative association between temperature and the winter peak of rotavirus infection in Kathmandu over the past decade (15-17, 21), it is essential to identify the minimum temperature range that poses a higher risk of infection among the exposed population. As observed in our findings, the risk of developing rotavirus infection is significantly higher at the lower quantiles (10th percentile as well as the 25th percentile) of the minimum temperature with reference to its median value. This observation implies that days with a minimum temperature between 4.2 °C to 7.8 °C represent high risk days for rotavirus infection among children in Kathmandu. Following this observation, to quantify rotavirus infection cases that could have been reduced with reference to the counterfactual scenario of lowest risk exposure (specific to minimum temperature), we used a backward attributable risk perspective and calculated attributable risk. From among the estimates of “dlnm” model, the lowest risk i.e., RR: 0.48 (95% CI: 0.29-0.78), corresponding to 21 °C (minimum temperature) was used as a reference value. We observed that the attributable fraction of rotavirus infection specific to minimum temperature exposure was 47.01%, which means that nearly half of the cases of rotavirus infection can be attributed to minimum temperature. This finding implies that, among the temperature indices used in our study, minimum temperature is an important predictor of rotavirus infection in Kathmandu. This information is crucial in minimizing the risk of rotavirus infection among children through the adoption of safe food handing and hand hygiene practices (29).

We observed an acute effect of temperature variation on rotavirus infection among children at different percentiles of all three indices of temperature. This finding may be related to the fact that rotavirus is a self-limited diarrhoeal illness (among immune competent hosts) that lasts only a few days and has a relatively short incubation period i.e., approximately 2 days, and usually less than 48 hours (29). Due to the unavailability of daily count data, we only used the weekly count of rotavirus infection cases, and the risk of rotavirus infection for different percentiles of temperature indices in our study was not significant at lag one or at subsequent weeks up to the fourth week (*Supplement figure S4*). Similar results indicating the acute effect of temperature on rotavirus infection have been reported in previous studies, including one from Nepal (6, 18, 19, 23). Atchison et al. (6), however, reported a lag effect of up to four weeks for the reported count of rotavirus, and an immediate effect for infection-rate parameters in their study conducted in the Netherlands and Great Britain. This prolonged lag effect could have been due to patients' inaccurate recall, or recall bias, regarding the precise date of diarrhoea onset when attending clinical facilities for treatment of diarrhoea (39). However, the biological plausibility (if any exists) behind the prolonged lag effect of temperature in their study remains unexplained.

Apart from temperature, we did not find a significant association between rotavirus infection and other meteorological variables i.e., mean relative humidity and total weekly rainfall. These findings mirror the results of studies from Great Britain, the Netherlands and Turkey (6, 35), and resonate with the findings of other epidemiological studies and laboratory based evidence (4, 34, 37). However, our findings did differ from those conducted in Australia (18), Bangladesh (19) and Hong Kong (22). Such differences could be due to geographical location, heterogeneities in socioeconomic conditions, demographic factors, behavioural factors or other environmental factors that may interact with climatic factors, complicating the dynamics of disease epidemiology at a local level (40).

The use of data from a single sentinel surveillance site may be a limitation of our study, as surveillance data only captures the proportion of cases seeking medical attention at the given facility. Hence, it is highly likely that rotavirus burden in Kathmandu is much higher, and the data analysed in our study may not necessarily represent all cases. Likewise, the use of weekly data spanning the period of four years for the purpose of a time series analysis may be another limitation of our study. We carried out subgroup analysis for gender, different age groups (stratified by months) and degree of dehydration to see if they are differentially associated with the exposure variables. Probably, due to the small sample size of our study, we could not find a significant association among gender, age groups and degree of dehydration with any of the exposure variables (maximum, minimum and mean temperature) used in the multivariate analysis (results not shown). Ideally, for trend analysis on the short-term effect of exposure-response relationships in environmental epidemiology, daily data for a considerably longer time period is desired. Rotavirus is a highly contagious disease with an increased likelihood of transmission from person-to-person contact. As such, failure to include a Susceptible-Infected-Recovered (SIR) model in our analysis may undermine the influence of host immunity on rotavirus transmission in our ecological model (41). A common problem associated with conducting epidemiological studies in low- and middle-income countries is the unavailability of updated information on demographic characteristics of the study population. The unavailability of this data, and other relevant health information, constrained us from conducting regression analysis using SIR parameters in our time series model. Despite these limitations, the major strengths of our study are the use of laboratory-confirmed cases on rotavirus infections furnished by a member laboratory of the Asian Rotavirus Surveillance Network, and the use of a distributed lag non-linear model for estimation of the exposure-response relationship.

In conclusion, our analysis demonstrated an inverse non-linear association between rotavirus incidence and all three indices of temperature, indicating a higher risk of infection during the cooler times of the year, and lower risk during the hotter times, in Kathmandu, Nepal.

Ethical consideration

The study was granted ethical approval by the Human Research Ethics Committee (HREC) of The University of Adelaide (H-2018-236), and Ethical Review Board of the Nepal Health Research Council (Reg. no. 560/2018). Data related to human participants used in the study were non-identifiable and were analysed anonymously.

Conflict of Interest

We declare no conflict of interest.

Abbreviations

AIC: Akaike Information Criterion; CI: Confidence Interval; DLNM: Distributed Lag Nonlinear Model; eCI: Empirical Confidence Interval; ELISA: Enzyme-linked immune sorbent assay; NS: Natural Cubic Spline; RR: Relative Risk; RT-PCR: reverse-transcription polymerase chain reaction; SIR: Susceptible-Infected-Recovered; WHO: World Health Organisation

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CRediT author statement

Dinesh Bhandari: Conceptualization, Research design, Methodology, Data curation, Software, Formal analysis, Visualization, Writing- Original draft; **Peng Bi** and **Scott Hanson Easey**: Conceptualization, Research design, Resource allocation, Validation, Supervision and Writing- Review and editing; **Jeevan Bahadur Sherchand** and **Meghnath Dhimal**: Data curation, Conceptualization, Research design, Supervision and Writing- Review and editing

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Figure 1: Trend of meteorological data and weekly Rotavirus infection among children under 5 years of age in Kathmandu Nepal.

Figure 2: Three dimensional plot and overall cumulative plot of risk ratio of rotavirus for maximum temperature (A), mean temperature (B) and minimum temperature (c).

Figure 3: Cumulative Risk Ratio of Rotavirus at different percentiles of temperature.

Table 1: Distribution of weekly average of meteorological data and Rotavirus infections among children under 5 years of age in Kathmandu, Nepal (2013-2016).

Study variables	Lowest	1st quartile	Mean	Median	3rd quartile	Highest
Maximum temperature (° C)	16.50	22.25	26.07	27.70	29.52	33.10
Mean temperature (° C)	7.90	14.80	19.52	20.40	24.30	26.10
Minimum temperature (° C)	-0.80	7.675	12.957	12.95	19.40	21.20
Relative humidity (%)	34.80	71.50	75.61	78.10	83.60	89.10
Rainfall (mm)	0	0	31.18	7.40	47.40	239.20
Rotavirus count	1	2	3	3	5	11

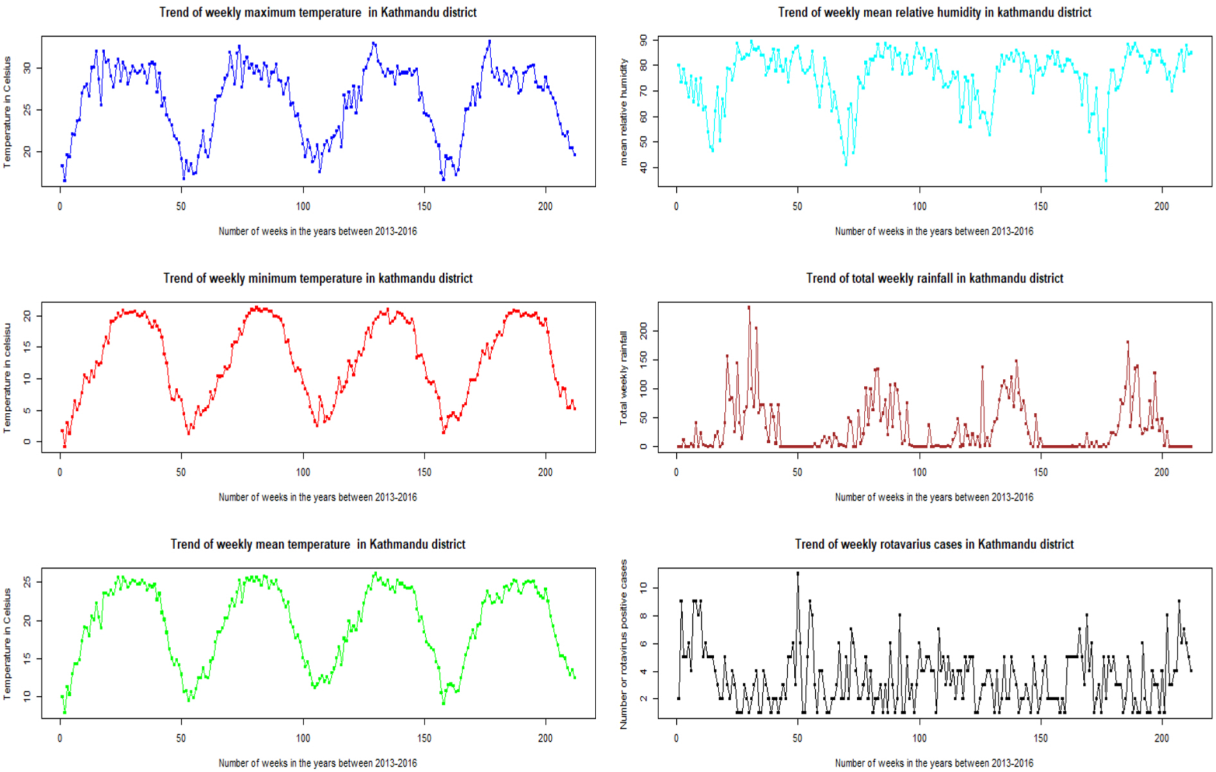
Table 2. Attributable risk (backward) of rotavirus infection among children (<5 years), specific to maximum temperature, mean temperature and minimum temperature, reported as attributable number and attributable fraction

Temperature indices	Total attributable number	Attributable Fraction [%] (eCI)
Maximum temperature	131	17.86(3.44 -27.62)
Mean temperature	319	43.59(13.62-64.80)
Minimum temperature	344	47.01(26.66-61.05)

Graphical abstract

Highlights

1. Rotavirus infection risk increased with decreased in all three temperature indices.
2. An inverse nonlinear association was observed between rotavirus infection and temperature.
3. Relative risk was higher for lower percentiles of temperature.
4. 47.01% of cases (<5 years age) were attributable to minimum temperature.
5. No significant association was observed with rainfall and relative humidity.



Trend of weekly maximum temperature in Kathmandu district

Trend of weekly mean relative humidity in kathmandu district

Trend of weekly minimum temperature in kathmandu district

Trend of total weekly rainfall in kathmandu district

Trend of weekly mean temperature in Kathmandu district

Trend of weekly rotavarius cases in Kathmandu district

Figure 1

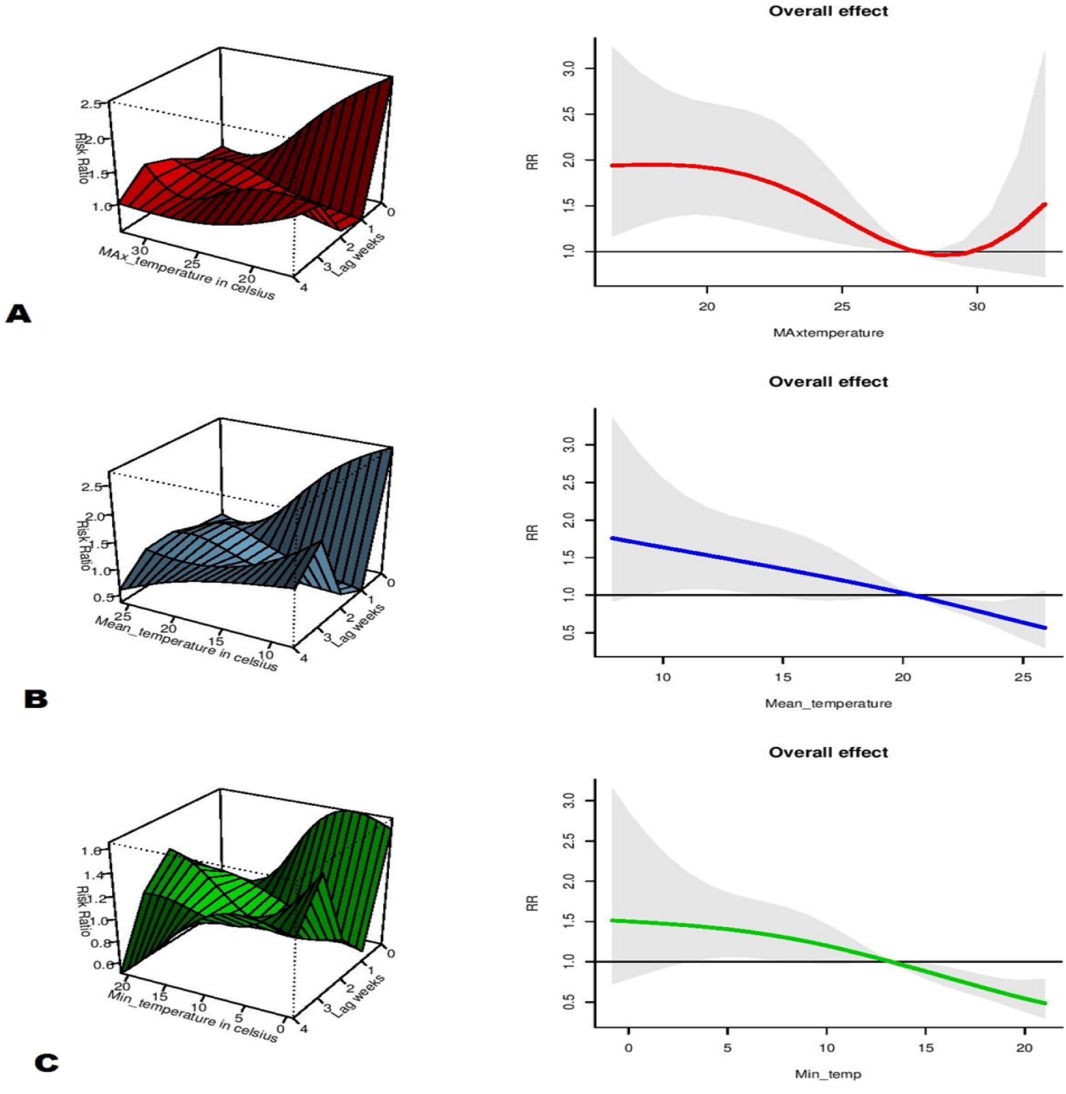

A
Risk Ratio
2.5
2.0
1.5
1.0
MAx_temperature in celsius
30
25
20
Lag weeks
0
1
2
3
4
Overall effect
RR
3.0
2.5
2.0
1.5
1.0
20
25
30
MAxtemperature
B
Risk Ratio
2.5
2.0
1.5
1.0
0.5
Mean_temperature in celsius
25
20
15
10
Lag weeks
0
1
2
3
4
Overall effect
RR
3.0
2.5
2.0
1.5
1.0
0.5
10
15
20
25
Mean_temperature
C
Risk Ratio
1.6
1.4
1.2
1.0
0.8
0.6
Min_temperature in celsius
20
15
10
5
0
Lag weeks
0
1
2
3
4
Overall effect
RR
3.0
2.5
2.0
1.5
1.0
0.5
0
5
10
15
20
Min_temp

Figure 2

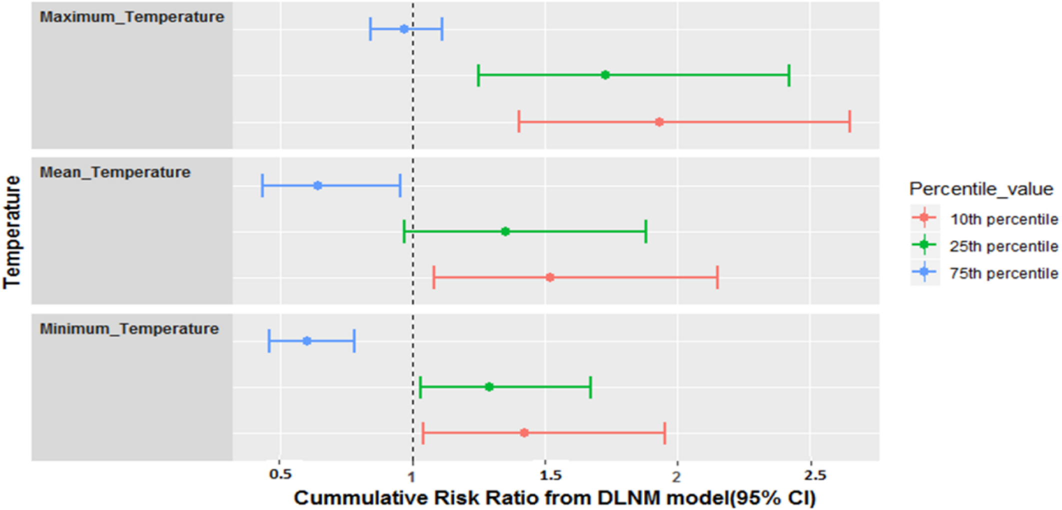

Maximum_Temperature
Mean_Temperature
Minimum_Temperature
Temperature
0.5
1
1.5
2
2.5
Cummulative Risk Ratio from DLNM model(95% CI)
Percentile_value
10th percentile
25th percentile
75th percentile

Figure 3