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Perennial malaria transmission and its association with rainfall at Kalahandi district of Odisha, Eastern India: A retrospective analysis

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Abstract. India contributes substantially to global malaria incidents. Vector dynamics is the significant determinant of malaria risk. Hence, knowledge on the interaction between rainfall, malaria cases and malaria vector density can be very useful for controlling malaria transmission. Kalahandi was screened for malaria cases, Anopheline vector density and their temporal relationship with rainfall. Epidemiological data was obtained from National Vector Borne Disease Control Programme, Odisha, India. Three years vector population study was carried out. Rainfall data was obtained from a database maintained by the Govt. of Odisha and was analysed using Univariate ANOVA and Pearson correlation co-efficient tests using R-prog. Malaria was found to be prevalent throughout the year attaining peak between July to August and another peak in December, amidst which the clinical malaria cases being recorded implied highest incidents in the month of July. The results estimated the seasonality of the population of *An. culicifacies*, *An. fluviatilis* and *An. annularis* over the region and determined the influence of rainfall on the vector population dynamics. Simple linear regression analysis suggested that at one month lag monthly rainfall (P=0.0007) was a significant meteorological factor. Rainfall seemed to be one of the best malaria predictors because of its positive correlation with proliferation of malaria cases in conjunction with *An. culicifacies* density making malaria a serious health issue in Kalahandi.

INTRODUCTION

Malaria is a major public health problem in India, contributing to a significant rate of human morbidity, mortality and economic adversity (Kumar *et al.*, 2007; Dhingra *et al.*, 2010). India contributes to about 49% of *Plasmodium falciparum* and 25% of *Plasmodium vivax* malaria cases amidst the approximate 145 million total malaria cases confirmed in South-East Asia (world malaria report (WMR), 2017).

Geographical advantage, climatic differences and ecological diversity make India an ideal place for breeding of a wide range of Anopheline vectors and to transmit malaria parasites (Das, 2012). The intensity of parasite transmission varies within different parts of the country (Singh *et al.*, 2012). The north-eastern, central and eastern states of India account for 80% of the total malaria incidents and deaths reported in the country and are observed as high malaria transmission zones (Annual report Health Govt, 2011; Patil & Kumar, 2011). Amongst these, the worst affected state is Odisha (Sahu *et al.*, 2011).

Odisha contributes approximately 3% of the population of India, and accounts for 43.6% of India's malaria cases, 58.2% of *Plasmodium falciparum* cases and about 33% of all reported deaths in 2016 (NVBDCP, 2017). In Odisha, the majority of the incidence and perennial transmission are reported at the Southern, Western and Northern belts among the 30 districts, which are substantially covered with forests and hilly areas. The average rainfall is 150cm, due to southwest monsoon during July to September with July being the wettest season when the major rivers may get flooded. Along with the retreating monsoon, the state also experiences small rainfall between October to November and January to February.

Climatic changes affect the geographic distribution of vectors and vector borne diseases and also increase the morbidity and mortality. Several climatic factors like temperature, rainfall, wind speed and relative humidity alter the life cycle of the mosquito and parasite development also human behaviour (i.e. location of hamlets, type of housing, sleeping habits, outdoor activities) and their poor knowledge about diseases seeking behaviour are of great significance as determinants of malaria transmissions (Sharma *et al.*, 2001; Upadhyayula *et al.*, 2015).

Rainfall play a vital role in malaria epidemiology by providing suitable breeding sites for the aquatic stages of the mosquito life cycle. In previous studies it has been showed that with the pattern of rainfall, temperature and humidity malaria incidence changes (VanDerHoek *et al.*, 1997; Haque *et al.*, 2010). In Africa, rainfall is considered to be a major factor influencing malaria cases (Abeku, 2007). Malaria cases were strongly associated with rainfall with a time lag of 0-3 months in Srilanka (VanDerHoek *et al.*, 1997). Natural disasters such as cyclones and floods may also have significant relationship with malaria outbreaks (Epstein, 2005). Additionally rainfall may increases relative humidity, above 60% which increases adult mosquitoes longevity and their vectorial capacity (Protopopoff *et al.*, 2009). Along with these ecological features, factors like tropical climatic conditions characterized by high temperature, humidity and medium to high rainfall provides the most favourable and useful environment for vector breeding and development of malaria parasite, thus making these region slightly vulnerable to malaria incidents (Evaluation LLIN, 2012).

In Odisha, 26 species of *Anopheles* mosquito have been recorded (Dash *et al.*, 1984). Presently in the entire state *An. culicifacies* and *An. fluviatilis* which are considered as the major vectors along with *An. annularis* as secondary vector (Ranjit, 2006). The vector density i.e. *An.culicifacies* density mainly increases during monsoon season in comparison to winter months, except in case of *An. fluviatilis* and *An. annularis* increases during winter (Baruah *et al.*, 2007).

Keeping in view of the unpredictability regarding the climatic change worldwide, this study was conducted taking different parameters such as rainfall, vector densities and epidemiology of malaria in Kalahandi district. The study was conducted to understand the association of rainfall with perennial malaria transmission in Kalahandi district, Odisha.

METHODS AND MATERIALS

Study area

Kalahandi is a district of Odisha in India (Figure 1), which is situated between 19.3N and 21.5N latitudes and 82.20E and 83.47E longitudes, has an area of 8,364.89 square kilometres. The topography of Kalahandi consists of plain land, hills and mountains. The climate of this district is quite extreme remaining mostly dry except during monsoon. The average annual rainfall of this district is 1378.20 mm without significant variation in rainfall between subsequent years. The monsoon starts from the end of June and generally lasts until September when the district receives its 90% of rainfall. Relative humidity is generally high from June to December with lowest (27%) during non-monsoon month March and highest (70%) during August.

Collection of data

Epidemiological data

Kalahandi district comprises of 16 Community Health Centre (CHCs) facilitating the diagnosis and treatment of malaria. The data on malaria were pooled on monthly basis which were collected from the CHCs. The epidemiological data sets of malaria cases from CHC level of Kalahandi district from the

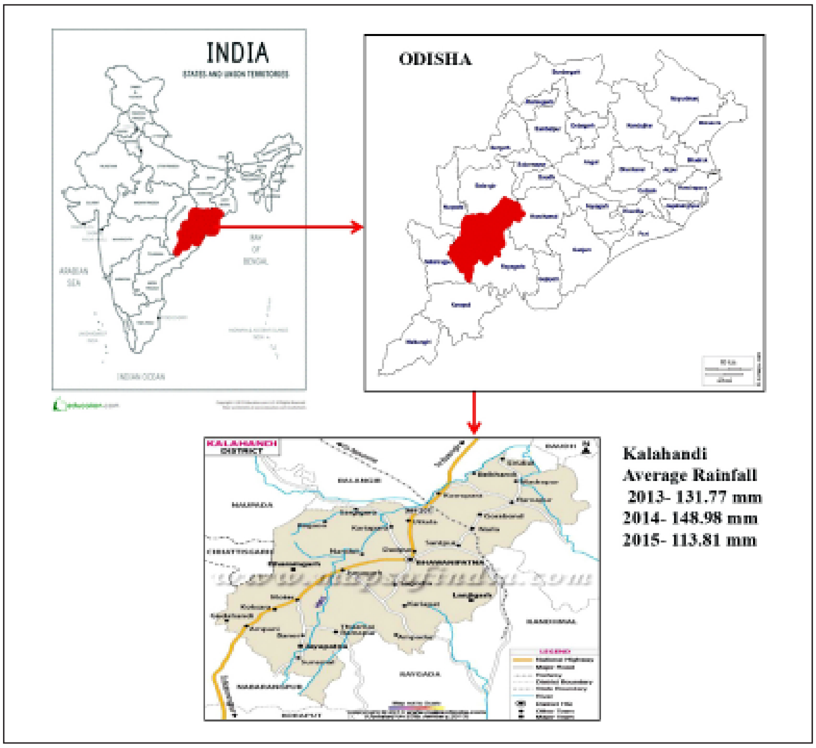

Figure 1. Map of Kalahandi district.

year 2013 to 2015 were obtained from NVBDCP, State Govt. of Odisha. The data includes malaria cases, Test Positive Rates (TPR), Annual Parasite Index (API) and Death cases.

Entomological data

The study was undertaken in 10 CHC (Jaipatna, Biswanathpur, Junagarh, Lanjigarh, M. Rampur, Narla, Th. Rampur, Kokasara, Parla and Karalamunda) of Kalahandi district. From each CHC two subcentres representing all ecotypes were selected and two villages were selected from each subcentre for routine entomological studies. In each village 5 fixed numbers of Human dwellings (HD), 5 fixed numbers of Cattle sheds (CS) and five random houses were selected in each visit.

Mosquitoes were collected from both the indoor and outdoor places with the help of mouth aspirator and torch during morning hour (6:00 to 9:00 am) and night hours (6:00 to 8:00pm) in the villages in each alternate month from January 2013 to December 2015 (Das *et al.*, 2013).

Anopheles mosquitoes were collected for 15 minutes from each dwelling. The mosquitoes collected in the field were labelled and brought to the laboratory for further identification and processing. The standard keys developed by Christophers (Christophers, 1933) was employed for the morphological identification of both the wild caught and adult mosquitoes reared from larval stage in the laboratory (Nagpal *et al.*, 2005). The number of vector mosquitoes collected per man hour is expressed as the vector density for the particular species.

Laboratory Processing

After identification, the head-thoracic portion and rest abdominal part of each mosquito were homogenized separately. Half of the homogenate from the head-thoracic region was dispensed to spot A and another half to spot B. Similarly, half of the homogenate from the abdominal part was dispensed to spot C and another half to spot B. Punched discs from these spots were then subjected to DNA isolation and multiplex PCR. Three vector species, *An. culicifacies* (n=150), *An.*

fluviatilis (n=150), *An. annularis* (n=150) were subjected to multiplex PCR for identification of sporozoites (*P. falciparum)* and blood meal intake (*H. sapiens*) using specific primer (Mohanty *et al.*, 2007; Rath *et al.*, 2015).

Meteorological data

Monthly meteorological data on rainfall were obtained from a database maintained by the Govt. of Odisha.(http://www.odisha.gov.in/disaster/src/RAINFALL/RAINFALL1/RAINFALL.html) (Meteorological data, 2016).

Statistical analysis

The number of malaria cases per month is used as a parameter for an indicator of malaria transmission. A correlation analysis was performed to relate the rates of malaria cases with the monthly rainfall (mm).

The R-prog version i386 3.4.1 statistical package was used for analysis. To compare the mean densities of *An. annularis*, *An. culicifacies* and *An. fluviatilis* and its densities between different seasons, One way ANOVA analysis was performed. Correlation analysis solved the purpose of understanding the relationship between rainfall and densities of *An. annularis*, *An. culicifacies*, and *An. fluviatilis*.

To determine the significant variation of TPR in 3 year One way analysis of variance (ANOVA) was used. A simple linear regression and Pearson correlation co-efficient (r) was run to analyse the association between the malaria cases, number of rainy day and rainfall.

RESULTS

Entomological Study

A total number of 14 *Anopheles* species were collected from both indoor and outdoor resting places. *An. culicifacies* was the predominant species among the vector species collected, followed by *An. annularis* and *An. fluviatilis*. Per man hour density (PMHD) of *An. culicifacies*, *An. fluviatilis* and *An. annularis* recorded during different months (Figure 2).

The PMHD of *An. culicifacies* ranges from 14.9 to 3.2. As per the data reported from the past 3 years, the density of *An. culicifacies* increases and peaked during August with the onset of Southwest monsoons starting from July and another peak found in April 2015. There was a positive correlation ($r = 0.44$, $P = 0.043$, $r>0$, $p<0.05$) between rainfall and density of *An. culicifacies*, which

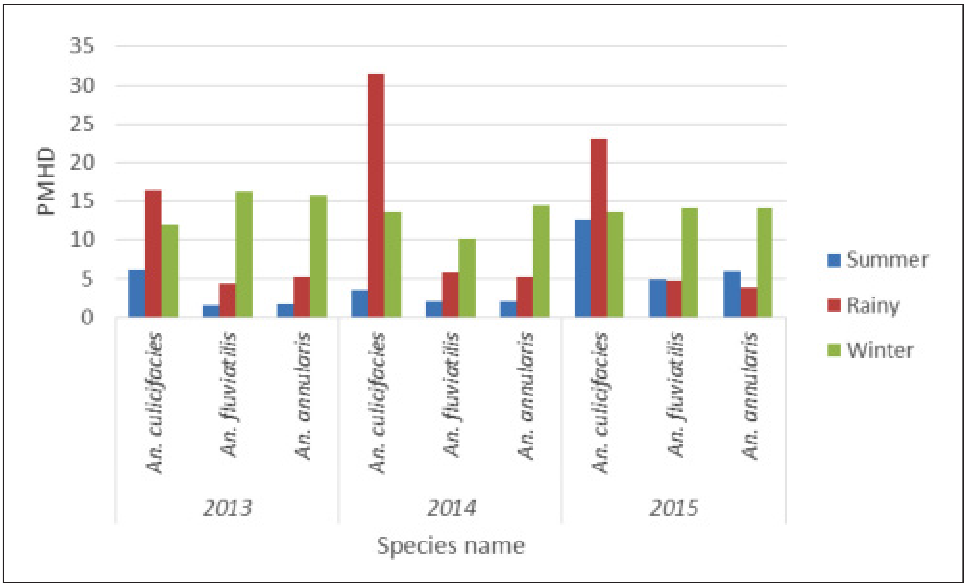

Figure 2. Per man hour density of *An. culicifacies*, *An. fluviatilis* and *An. annularis* in Kalahandi district during the 3 seasons.

showed that it was statistically significant (Figure 3). However, the PMHD of *An. culicifacies* was found to be more in rainy seasons than winter and summer seasons, but the difference was statistically not significant.

The PMHD of *An. fluviatilis* was recorded lowest in June (summer month) and peaked during December and February (winter months). No relationship was observed for the density of this species with that of the rainfall ($r = -0.27$; $P = 0.8$).

The PMHD of *An. annularis* was lowest in June and August (summer and rainy months), increased with the onset of pre winter in October, and peaked during December and February (winter months). Alike *An. fluviatilis*, the density of *An. annularis* also did not show any significant relationship with rainfall ($r = -0.22$; $P = 0.81$). No significant difference was found in *An. fluviatilis* and *An. annularis* density between the three seasons (Figure 2).

Molecular Work

The multiplex PCR results revealed that, 77 specimens, 93 specimens and 84 specimens of *An. culicifacies*, *An. fluviatilis* and *An. annularis* respectively were found positive for human blood with a sporozoite rate of 0.57%, 0.41% and 0.18% respectively. *An. culicifacies* sporozoite rate was higher in the rainy season than the winter season in both the year 2013 and 2015. However, sporozoite rate of *An. fluviatilis* and *An. annularis* was higher in winter season (Figure 4).

Annual variation of the Malaria cases and Rainfall in Kalahandi district from 2013 to 2015

Considering the observation period of 3 years, monthly malaria cases were reported representing distinct peak period for different seasons. The highest malaria cases were recorded in July 2015. The peak periods were recorded predominantly between July and August and another peak recorded in the month of December. Peaks in malaria cases found in the year August 2015 followed by moderate peaks of rainfall (286.2 mm) occur in 28 days and in that month density of *An. culicifacies* (14.9) was also high while declines in malaria cases (1344 cases) were associated with high peaks of rainfall i.e 594.2 mm occur in 23 days, found in the year June 2013 and in that month density of *An. culicifacies* (3.3) was less.

The test positive rate (TPR) was recorded highest in the month of August 2014 and August 2015 followed by 408.3 mm and 286.2 mm rainfall (Figure 3) respectively. There was a sharp decline in total positive rate between July 2013 and August 2013 followed by an increase between July 2014 to August 2014. A slight increase in total positive rate was recorded between July 2015 and August 2015.

One way ANOVA revealed that there was no significant annual variation among malaria cases ($F=13.08$, $P>0.001$) (Figure 3) and Total malaria positive cases ($F=3.5$, $P>0.001$).

In the years 2013 to 2015, change in the pattern of rainfall was recorded with a maximum annual rainfall (148.98 mm) in 2014 and minimum annual rainfall (113.81 mm) in 2015. However, there was no significant annual variation found in rainfall ($F=13.08$, $P>0.001$) as depicted by One way ANOVA.

Relationship between Malaria cases, Rainfall and Rainy days

Simple linear regression between Rainfall (independent variable) and Malaria cases (dependent variable), Kalahandi district, 2013-2015

Regression analysis aims towards estimating or predicting the occurrence of malaria cases and its association with rainfall variable. Simple linear regression analysis was done at the same month and one month delay (Table 1), and the results obtained showed that rainfall within the same month was negatively correlated with malaria cases. At one month delay rainfall ($P=0.0007$, $p<0.001$) was a statistically significant meteorological factor for monthly malaria cases.

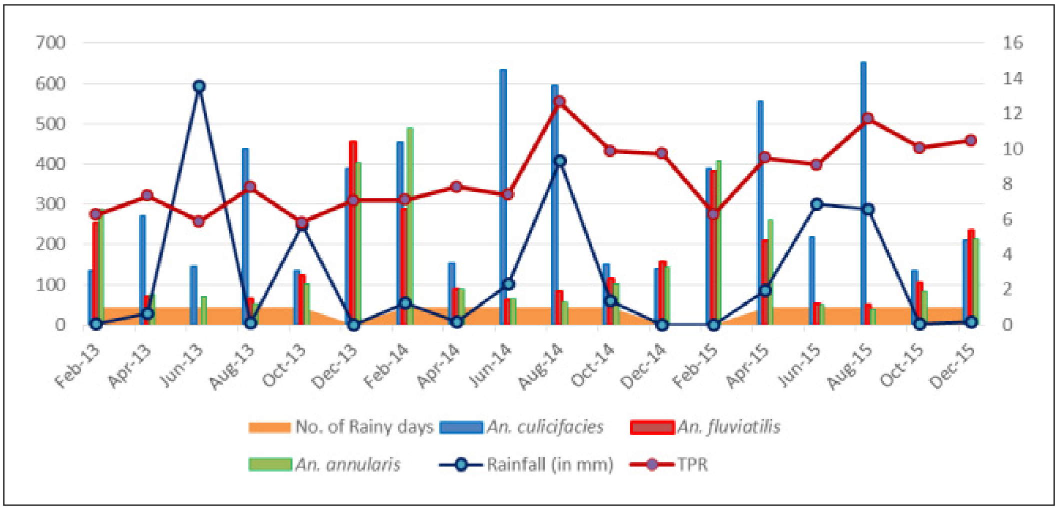

Figure 3. Monthly abundance of *Anopheles* vector species and their relation among rainfall, no. of rainy days and malaria morbidity in Kalahandi district.

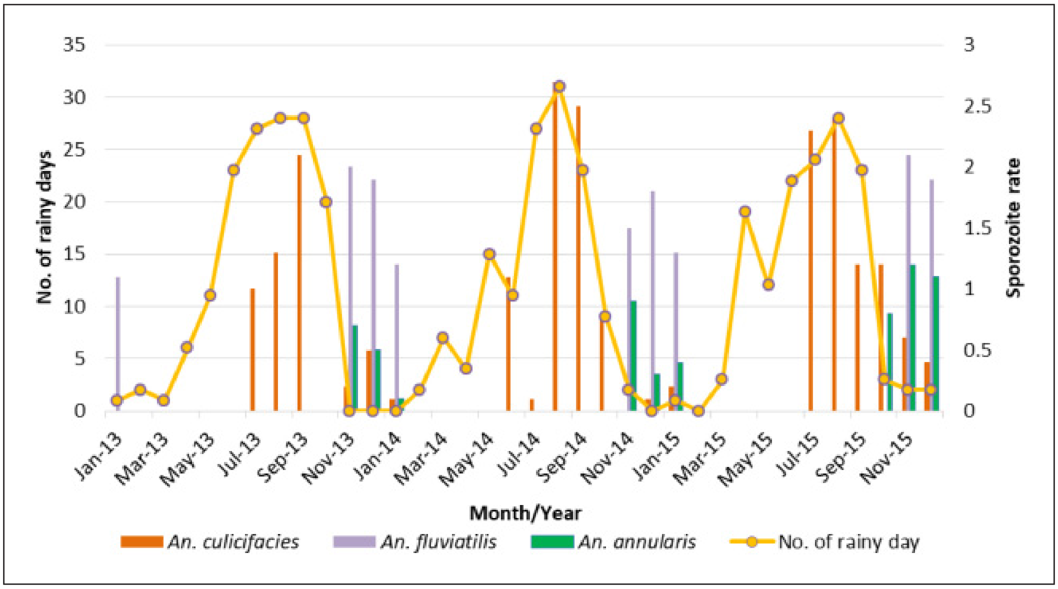

Figure 4. *P. falciparum* sporozoite rates and their relation with no. of rainy days in Kalahandi district.

Table 1. Simple linear regression between rainfall and monthly clinical malaria cases in Kalahandi district, 2013-2015

	β Coefficients	R^2	F	P-value	Lower 95%	Upper 95%
Rainfall	2.3	0.20	8.6	0.005	0.72	3.97
One month lag Rainfall	2.7	0.44	13.4	0.0007	1.27	4.32

Pearson correlation analysis between rainfall and malaria cases, Kalahandi district, 2013-2015

To observe the strength of association between malaria cases, no. of rainy days and rainfall, a Pearson correlation analysis was performed. Malaria cases were positively associated with rainfall and number of rainy days with a P value = 0.002(rho=0.46, 95% CI 0.2162 to 1.00) and P value = 0.001(rho=0.47, 95% CI 0.224 to 1.00) respectively.

DISCUSSION

This study draws an attention on the interaction between rainfall, malaria cases and malaria vector density. It is important to study the impact of rainfall on the transmission of malaria, which may directly or indirectly influence the mosquito density (Wu *et al.*, 2007).

This study was undertaken in a hilly, forested and plain area of Kalahandi district, which has been hyperendemic for *P. falciparum* malaria, its API was 13.17, 19.14 and 20.13 in 2013, 2014 and 2015 respectively. We found perennial malaria transmission in Kalahandi district due to highest abundance of *An. culicifacies* throughout the rainy season (July and August) followed by summer season (April), similarly the winter seasonwas also abundant with *An. fluviatilis* and *An. annularis* vector species. This may be due to the existence of perennial streams and the preferential breeding habitat *where* these species play a major role in transmission of malaria. In our study, the principle malaria vector *An. culicifacies* was found positively associated with rainfall. *An. culicifacies* breeds in rice fields (Dash, 1984) seem to be positively associated with rainfall. Rainfall affects the malaria transmission as it modifies the temperature by increasing the relative humidity and also affects the site and abundance of mosquito breeding (Pampana, 1969). Relative humidity affects the activity and survival of *Anopheles* mosquitoes, thereby enhancing malaria transmission. The peak incidence seasons (July to October) of malaria cases which coincides with the rainy season in the state is may be occurred due to the increase in breeding places of different vector species to different types of water bodies (Nagpal & Sharma, 1995). From the present study, it was ascertained that the transmission of the disease occurred throughout the year, with the higher number of cases being recorded mainly in rainy/wet (July, August) followed by winter/cold (November, December) and summer/dry (March/ April).

In Arunchal Pradesh and China, an increase in the density of parasites was observed with the start of the early monsoon in May reaching first peak in July. It was also confirmed that *P. vivax* and *P. falciparum* are the causatives of high malaria cases depending mostly upon the climatic factor types (Zang *et al.*, 2010; Mutheneni *et al.*, 2014). In our study, parasite density rises soon after the start of the early monsoon in July attaining first peak in August, as adequate breeding sites for mosquito vectors were created by the rain water. During the short rains, when survival rates were lowest, the sporozoite rate of *An. culicifacies* was 0.62. Although the survival rates of *An. fluviatilis* and *An. annularis* rose during the dry season and few were found to be positive. Transmission of infection subsequently rises as vector population increases; hence, there is an increase in parasite densities. Decrease in overall rainfall, at the end of the August the breeding grounds dries up which causes decrease in vector density and it correlate with parasite density which falls abruptly reaching a minimum in October. Further a second peak in mean parasite density observed in the month of November and December, because of the density of *An. fluviatilis* and *An. annularis* which is high in winter and slowly a decline trend in parasitic density was observed.

In our study, moderate rainfall was suitable for *An. culicifacies* vector abundance. Due to moderate rainfall microhabitat is increased. These conditions increase the longevity of adult mosquitoes by prolonging vector life span. In high and low rainfall with less number of rainy days, we found less vector density and less malaria cases. All in all, there is a general consensus that rainfall can influence malaria transmission either positively by creating suitable habitats or negatively by flushing breeding sites depending on its intensity. When excess rain occurs in a critical months, the risk of an epidemic is greater in a year. Simple linear regression analysis also revealed a negative correlation of maximum rainfall at zero month effect with the malaria cases, which might be due to much rainfall that washes out the breeding sites of the vectors and affects the life cycle of mosquito. We established the correlation between malaria cases and rainfall at one month lag periods and found positive relation (P= 0.0007, $p<0.001$). Though rainfall is the major factor to enhance malaria transmission (Thomson *et al.*, 2005), some studies shown negative and moderate correlation of malaria cases with rainfall (Bhattacharya *et al.*, 2006; Bashar & Tuno; 2014). We also established the Pearson correlation and found moderate relation (r=0.46, P=0.002), (r=0.47, P=0.001) between the number of malaria cases and rainfall and number of rainy days respectively. Rainfall plays an important role in enhancing survival chances of vector mosquitoes and associated with plasmodium parasites during early and post rainy season. Galardo et al. suggested that if sufficient biological and seasonal patterns of the vectors are known then rainfall could be used to predict vector abundance (Galardo *et al.*, 2009).

CONCLUSIONS

The study demonstrates that malaria incidences continue to occur all-round the year in Kalahandi district due to favourable climatic conditions. It was observed that rainfall played a key role in the transmission of malaria. Rainfall is significantly associated with clinical malaria transmission and positively correlates with *Anopheles* vector densities. Therefore, to reduce morbidity and mortality in Kalahandi district, an integrated vector management system should be covered round the year. Additionally, primary and applied research on the social, ecological and economic aspects of the disease is required for the regular evaluation of malaria situation in an endemic area.

Limitation of the study

In this study we found rainfall positively associated with malaria cases. More factors like temperature, humidity and non-climatic factors like human behaviour are playing a vital role for perennial transmission of malaria in Kalahandi district. This study did not include other parameter such as temperature, humidity etc. due to unavailability of data, which may be important considerations for an early warning system.

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Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this study.

Ethical Approval

Not required.

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