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SEASONAL DISTRIBUTION OF *ANOPHELINE* SPECIES AND THEIR ASSOCIATION WITH METEOROLOGICAL FACTORS IN PUNJAB, PAKISTAN

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ABSTRACT

The relative abundance of Anopheline species and its relationship with various climatic variables were investigated in Kasur and Shiekhupura districts of province Punjab, Pakistan. Anopheline mosquitoes (n=4973) were captured during the period of November, 2009 to October, 2010. Total six Anopheline species were identified from different localities of both districts. Results shown high relative abundance of *An. stephensi* (62.5 %) followed by *An. subpictus* (25.7%), *An. culicifacies* (5.48%), *An. pulcherrimus* (3.65%), *An. annularis* (1.34%) and *An. nigerrimus* (1.16%) respectively. The month-wise, highest abundance of Anopheline species was seen in the month of July (19.26%) and lowest in December (0.70%). The seasonal abundance of mosquitoes in both districts was higher in the rainy season followed by high abundance at the start of the post monsoon period, i.e. in the months of September (17.21%) and October (13.71%). While there was a decline in the abundance of mosquitoes during the late monsoon period due to decrease in the rainfall and temperature. Moving towards the end of the dry period, it is shown again increase in the surge in mosquitoes i.e. March (4.92%) to June (15.38%) due to increase in rainfall. The results revealed that the population dynamics of Anopheline mosquitoes vary with the change in geographic boundaries, demographic and climatic conditions.

Key Words: Anopheline species, Monthwise abundance, Seaonal abundance, Kasur, Shiekhupura.

INTRODUCTION

Malaria is endemic in 109 countries, including Pakistan. It is one of the most formidable and devastating diseases of the world. Globally, about 3.3 billion people live under the threat of Malaria (WHO 2008, 2012). Since the discovery of the malaria parasite and establishment of mosquito's role in transmitting malaria, it continues to be a major global health problem (Dash *et al.* 2007). In Pakistan both *P. falciparum* and *P. vivax* are reported along with mixed infection cases of both as one of major health problems (Beg *et al.* 2008; Raza *et al.* 2013; Yasinzai and Kakarsulemankhel 2008)

All over the world, mosquitoes belonging to genus *Anopheles* play integral role in transmission of human malarial protozoa. Worldwide about 465 recognized Anopheles species and more than 50 unnamed members of Anopheles species complexes are reported, of which about 70 have the capacity to act as vectors for Plasmodium (Marianne 2013; Sinka *et al.* 2012; WHO 2008). Among these about 23 Anopheline species have also been reported in Pakistan. Of which 2 Anopheline species have been confirmed as malaria vectors i.e. *An. culicifacies* as primary vector in rural areas and *An. stephensi* as important vector in urban areas of the country (Ilahi and Suleman 2013; Jahan and Hussain 2011).

The meteorological factors directly affect the qualitative and quantitative alterations in mosquito breeding sites. Parameters like rainfall, temperature and relative humidity are mainly responsible for seasonal variations. These environmental changes may regulate adult mosquito populations and transmission pattern of various mosquito borne diseases including malaria (Bashar and Tuno 2014; Wongkoon *et al.* 2013). The vector capacity of a mosquito is directly regulated by its species relative abundance and local climatic conditions (Roiz *et al.* 2014).

The present study provides the relationship between various climatic variables and the relative abundance of Anopheline species in Kasur and Shiekhupura districts of Punjab, Pakistan.

MATERIALS AND METHODS

Study Areas: Two districts of Lahore division (i) Kasur and (ii) Shiekhupura in Punjab province, Pakistan were selected for the present study. These districts were selected on the basis of being intensive rice irrigation areas, highly malarial endemic and the suitable climatic conditions for mosquito breeding.

District Shiekhupura is situated at 31. 71º North Latitude, 73.98º East Longitude and elevated at about 214 meters above the sea level. On the north it is bounded by two districts i.e. Gujranwala and Hafizabad, on the North-East by district Narowal, on the West and South West by district Nankana Sahib and on the East it is bounded by district Lahore. It is occupying about 5,960

Km² area with about 857,000 inhabitants. The climate is subject to extreme variations with a dry season from March to June, monsoons from end of June to about mid September and post monsoon and winter from the end of September to February. The average temperature ranges between 20-31ºC, average relative humidity about 46 % and 44 mm of average annual rainfall.

District Kasur is about 55 km towards the South of Lahore situated at 31. 12º North Latitude, 74.45º East Longitude and elevated at about 201 meters above the sea level. On the north it is bounded by district Lahore, on the North-East by district Shiekhupura, on the South West by district Okara, on the South by Indian Territory and across the river Sutlej. It spreads over an area of about 3,995 Km² with about 2,918,000 inhabitants. The climate is hot in summer and comparatively cold in winter. May and June are the hottest months. The average temperature ranges between 19-31ºC, average relative humidity about 57% and 62 mm of average annual rainfall.

Mosquito's collection and morphological identification: The entomological survey of Anopheline species in these districts was conducted to compile baseline data. It was a descriptive observational study. For this purpose, adult mosquito specimens were collected from different localities of districts Kasur and Shiekhupura.

From November, 2009 to October, 2010, after approved consent of the villagers, by using convinent random sampling fifteen villages were selected from various Tehsils of each district on the basis of previously available data regarding malaria endemicity and according to feasibility of work in these villages. Anopheline mosquitoes (n=4973) were collected fortnightly every month from human dwellings and animal sheds from these selected villages throughout the year. In each village six human dwellings and three animal sheds were selected for collection. As per mosquito behavior dawn and dusk timings were selected for collection.

Mosquitoes were collected by using hand catch techniques. For this purpose resting mosquitoes on different surfaces were collected by using both mouth aspirator and mechanical aspirator. CDC sweeper was also used in areas/ surfaces where the rich density of mosquitoes was found (WHO 1975). After collection mosquitoes were transferred in disposable mosquito cages. Mosquitoes were transported to Department of Parasitology, University of Veterinary and Animal Sciences (U.V.A.S.), Lahore for further identification. Each cage was covered with a water soaked cotton swab and was provided with 10% sucrose solution.

After field collection, mosquitoes were identified up to the species level under CO_2 anesthesia using a stereoscope according to key for morphological identification being provided by (Amerasinghe *et al.* 2002). After species identification, Anopheline mosquitoes were selected for the further study. Anopheline species were preserved for morphological identification only and complete record was tabulated.

Meteorological Data: Data regarding average rainfall, relative humidity and temperature of the study areas, from November, 2009 to October, 2010 were collected from the local stations of the meteorological department of Punjab, Pakistan.

Statistical Analysis: In a descriptive analysis, mean and standard deviation (SD) were calculated. All analyses were carried out using Microsoft Excel 2007 and statistical software Graphpad Prism and Graphpad Instat version 3.10.

RESULTS

During the present entomological survey, a total of 4,973 Anopheline mosquitoes were captured. These were further differentiated up to the species level on the basis of their morphological features. Total of six Anopheline species were identified from different localities of both districts. Results show overall high relative abundance of *An. stephensi* (62.5%) followed by *An. subpictus* (25.7%), *An. culicifacies* (5.48%), *An. pulcherrimus* (3.65%) *An. annularis* (1.34%) and *An. nigerrimus* (1.16%) respectively (Table 1.1). The P>0.05 shown no significant variation among Anopheline species captured from both districts.

Table 1.1. Distribution of Anopheline species.

Districts/ Anopheline Species	Mosquitoes Captured		
	Kasur n(%)	Shiekhupura n(%)	Total n(%)
An. annularis	36(1.3)	31(1.3)	67(1.34)
An. culicifacies	132(5.0)	141(5.9)	273(5.48)
An. pulcherrimus	90(3.4)	92(3.8)	182(3.65)
An nigerrimus	27(1.03)	31(1.3)	58(1.16)
An stephensi	1,635(63.6)	1,476(62.4)	3,111(62.5)
An subpictus	690(26.4)	592(25.0)	1,282(25.7)
Grand Total	**2610(52.48)**	**2363(47.52)**	**4973**

Monthwise prevalence of Anopheline species: The month wise relative abundance of Anopheline species was also determined. The highest abundance of Anopheline species was seen in the month of July (19.26%) and lowest in the month of December (0.70%). The month wise percentage values and overall means with standard deviations of Anopheline species are shown in (Table 1.2). The P<0.05 shows that variation among month wise abundance of mosquitoes differs significantly.

The percentage values of comparative abundance of Anopheline species in both districts had shown almost the same pattern of mosquito abundance in both districts. The highest abundance was seen in the month of July in both districts (19.11 % and 19.42% in districts Kasur & Shiekhupura); while lowest abundance in both districts was seen in the month of December (0.57% and 0.84% in districts Kasur and Shiekhupura respectively). (Fig 1.1).

Table 1.2. Means ± SD of month wise Anopheline species captured

Months	Mean ±SD		
	Total	Kasur	Shiekhupura
November	8.0±9.5	3.4±4.3	4.5±5.5
December	5.8±8.1	2.5±3.3	3.3±4.9
January	17.0±29.1	8.0±13.3	9.0±16.0
February	25.8±43.1	13.8±24.5	12.0±18.8
March	40.8±69.7	19.8±32.1	21.0±37.7
April	45.6±78.0	24.2±43.4	21.5±34.8
May	29.6±44.2	14.3±19.6	15.3±24.8
June	127.5±255.7	74.3±156.0	53.2±99.8
July	159.6±301.9	83.2±161.5	76.5±140.5
August	112.5±135.5	53.3±64.0	59.1±71.8
September	142.6±166.9	77.3±95.7	65.3±72.9
October	113.6±148.9	60.7±83.1	53.0±67.5

The highest value for both districts was seen in the month of July (i.e. 83.2±161.5 and 76.5±140.5 for districts Kasur and Shiekhupura respectively). While the lowest values of means ± SD were seen in the month of December for both districts (i.e. 3.4±4.3 and 4.5±5.5 for districts Kasur and Shiekhupura respectively). The $P<0.05$ shows extremely significant variation among total month-wise abundance in both districts

Seasonwise prevalence of Anopheline species: The present study revealed that the overall abundance of mosquitoes in both districts was higher in the rainy season, especially in the month of July (19.26%) followed by high abundance at the start of post monsoon period i.e. in the months of September (17.21%) and October (13.71%). While there was a decline in the abundance of mosquitoes during the late monsoon period due to decrease in the rainfall and temperature with the lowest abundance in the month of December (0.7%). While moving towards the end of the dry period, it shows again increase in the surge of mosquitoes i.e. March (4.92%) to June (15.38%) due to increase in rainfall and followed by the start of monsoon period.

The comparison of meteorological data with the month-wise relative abundance of Anopheline species in both districts Kasur and Shiekhupura respectively has shown in (Figs 1.2 & 1.3). The data interpretation from each district exhibited the same pattern of mosquito's abundance as mentioned above with highest abundance in rainy season followed by decline in the late monsoon period and lower in the winter season. While by the start of the summer season there was again rising in the mosquito population due to changes in average rainfall and mean temperature.

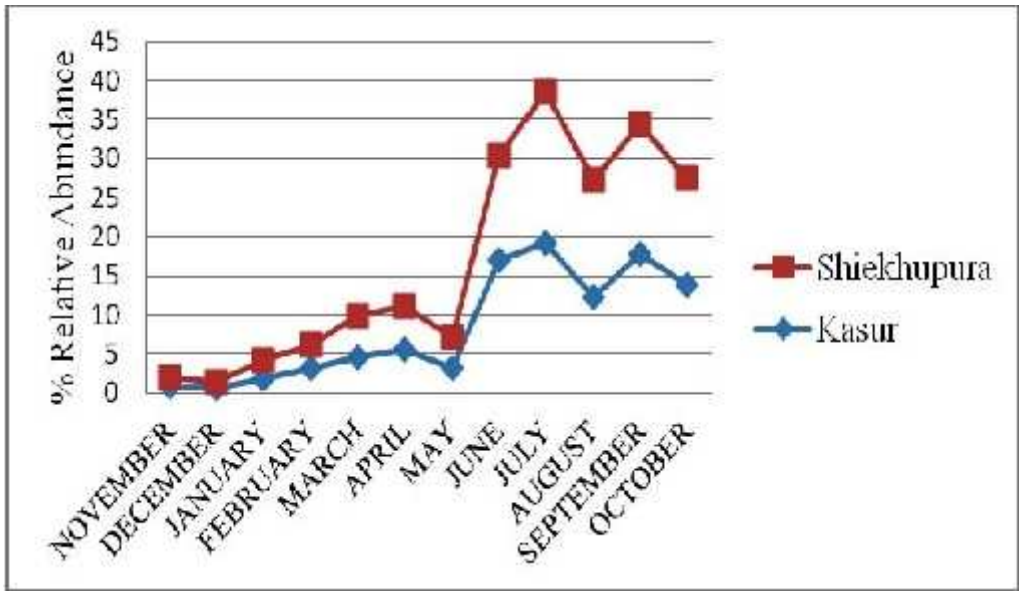

Figure 1.1. Month wise comparative distribution of Anopheline species

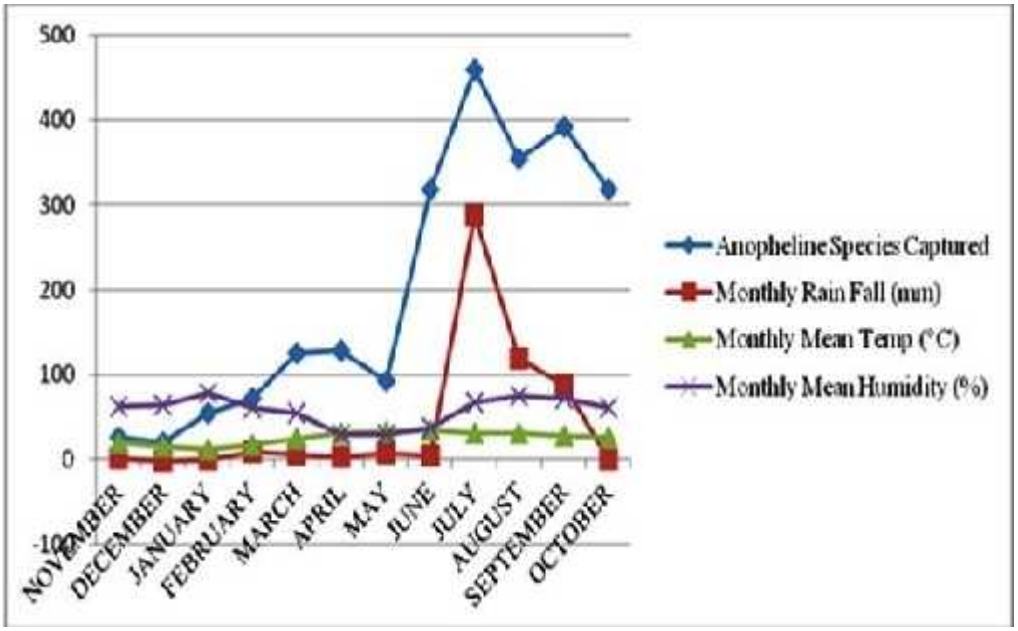

Figure 1.2: Comparison of meteorological data with relative abundance of Anopheline species in district Kasur

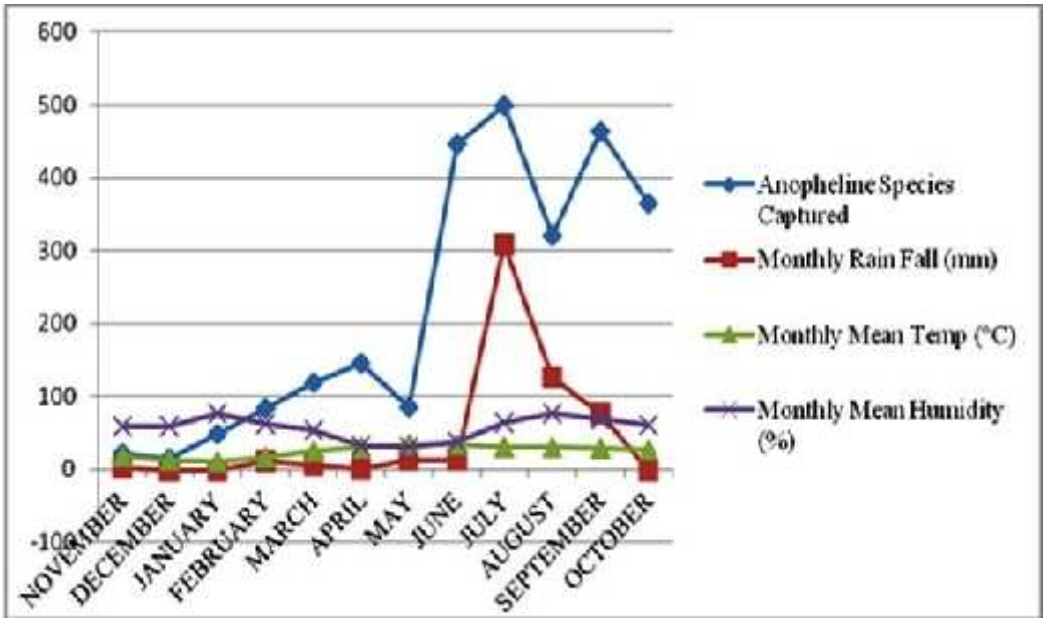

Figure 1.3: Comparison of meteorological data with relative abundance of Anopheline species in district Shiekhupura

DISCUSSION

For effective control of a disease sound knowledge about its vector epidemiology is one of the key components. Based on this statement this survey was conducted to investigate about the relative abundance of Anopheline species in districts Kasur and Shiekhupura. Total n=4973 mosquitoes captured were belonging to genus Anopheles the only genus responsible for transmitting all five species of Plasmodium in human beings. In 1971 the first checklist of the mosquitoes of Pakistan enlisted about 38 Anopheline species in the country which reduced to 22 after separation of East Pakistan (now Bangladesh). Later in 2002 the updated key of Anopheline species in Pakistan reported about 24 Anopheline species. But in the present survey, we found only six species as a whole from both districts. These include *An. annularis*, *An. culicifacies*, *An. nigerrimus*, *An. pulcherrimus*, *An. stephensi* and *An. subpictus* (Table 1.1). All these species belong to already known Anopheline species in Pakistan. These 6 species had been also reported in different districts of Punjab in 1980

(Amerasinghe *et al.* 2002; Khan 1971; Rathor *et al.* 1980; Sinka *et al.* 2012).

In one study conducted in Peshawar, Pakistan they identified 6 Anopheline species from which 4 were also found in present study i.e. *An. culicifacies, An. nigerrimus, An. stephensi* and *An. subpictus* while *An. annularis* and *An. pulcherrimus* were not found in the present studyse. Whereas *An. fluviatilis* and *An. maculatus* found in an earlier study (Ali and Rasheed 2009). Similarly, in district Muzaffargarh Southern Punjab, Pakistan five Anopheline species were captured among which 3 species i.e. *An. culicifacies, An. Stephens* and *An. subpictus* were also identified in the present study. While 2 species, i.e. *An. fluviatilis* and *An. superpictus* were not found in districts Kasur and Shiekhupura (Rana *et al.* 2014). One other study occurred in South Punjab also reported the prevalence of *An. culicifacies, An. pulcherrimus, An. stephensi* and *An. subpictus* in the area (Herrel *et al.* 2001).

In one another study in Swat, Pakistan in addition to *An. annularis, An. culicifacies* and *An. stephensi*, they also reported *An. bitaeniorhynchus, An. dthali, An. fluviatilis, An. lindesayi, An. maculatus, An. pallidus, An. pseudovishuni,* An. *quinquefasciatus* and *An. splendidus*. A noticeable difference can be seen in the species composition studied in Swat and in Kasur and Shiekhupura. This might be because of various reasons:

a) Difference of sampling techniques being adapted in these two studies. We only captured mosquitoes through hand catch technique. While they in addition to this technique also used total catch and various bait traps to capture mosquitoes.
b) Secondly, we only focused on the collection of adult mosquitoes, whereas they also collected various immature stages of mosquitoes as well.
c) There is a major difference in the geography and climatic conditions of the areas studied. The Swat belongs to Province Khyber Pakhtunkhwa (previously NWFP) is a mountainous area with cool and refreshing climatic conditions in the upper parts of the area. Whereas Kasur and Shiekhupura belong to the hottest parts of Province Punjab. The average rainfall in Swat is also much higher than in these 2 districts (Ali *et al.* 2013; Ilahi and Suleman 2013).

Among these captured Anopheline species only *An. culicifacies a*nd *An. Stephens a*re reported as major malaria vectors in the country. *An. culicifacies i*s mainly known as primary malaria vector, especially in rural areas while *An. stephensi a*s secondary malaria vector mainly in urban areas and secondary in rural areas of the country (Ali *et al.* 2007; Husain and Talibi 1956; Mahmood *et al.* 1984; Reisen *et al.* 1982). *An. culicifacies* is now referred as a species complex comprising of 5 isomorphic species (A, B, C, D and E). These isomorphic species are not identified on a morphological basis, that's why we also only identified it as single species i.e. *An. Culicifacies (*Barik *et al. 2009;* Marianne *2013;* Sinka *et al. 2011).*

The findings of the present study vary from the above mentioned facts. Relatively higher abundance of *An. stephensi* (63%) followed by *An. subpictus* (5.4%) was found even from rural areas of Kasur and Shiekhupura and only (5.4%) mosquitoes were identified as *An. culicifacies* out of total Anopheline species (Table 1.2). These results are in accordance with the findings of study related to malaria vectors of the Southern Punjab. They also reported a major shift in the malaria vector species composition over the last 3 decades. They also found a relative increase in the abundance of *An. stephensi* to *An. culicifacies*, which was highly prevalent in 1980's (Klinkenberg *et al.* 2004; Reisen *et al.* 1982). Our findings are also being supported by the results of one another study conducted in NWFP Province regarding incidence of *Plasmodium falciparum* malaria. They reported peak transmission of *P. falciparum* malaria in the month of October when *An. culicifacies w*as completely disappeared while *An. stephensi w*as still prevailing (Rowland *et al.* 2002). Similar changes in the relative abundance of these two malaria vectors were also observed in different studies (Ali *et al.* 2013; Herrel *et al.* 2004; Ilahi and Suleman 2013). The low density of *An. culicifacies a*nd high density of *An. stephensi i*s the plausible explanation that *An. stephensi m*ay impart important role in the transmission of *P. falciparum* malaria in these areas.

An. subpictus was the second highly abundant species (26%) found in the present study in both districts (Fig 4.4). An increase in the surge of *An. subpictus* was seen in the late monsoon and early post monsoon season, which was opposite to the surge of *An. stephensi* which showed declines in these seasons. Whereas the transmission pattern of *P. falciparum* malaria in Kasur and Shiekhupura shows increase in the incidence of cases in late monsoon and early post monsoon periods. These results may propose about the possible role of *An. subpictus* in the transmission of *P. falciparum* malaria. This mosquito is already well defined as a malaria vector in different countries, including neighboring countries of Pakistan (Chandra *et al.* 2010; Elango *et al.* 2011; Kumari *et al.* 2009; Sinka *et al.* 2011). The vectorial capacity of *An. subpictus* for *P. falciparum* is not well defined in the country. In some studies, it is suspected as a malaria vector in different regions of the country (Mahmood *et al.* 1984; Rana *et al.* 2014). Hence, in this scenario further studies should be planned to exactly know about the role of *An. subpictus* in malaria transmission in Pakistan.

The result of present study reflects the influence of seasonal fluctuations on the population dynamics of different Anopheline species (Fig 1.1). This variation also affected the transmission pattern of *P. falciparum* malaria in Kasur and Shiekhupura. Meteorological data reveal that variations in temperature, mean evaporation and

average rainfall in different months are mainly responsible for this change (Fig 4.36). The reciprocal effect of high temperature and low rainfall was seen in the seasonal abundance of Anopheline species in our study. The highest prevalence of Anopheline species was seen in the month of July followed by September (Table 4.5). These results are in accordance with the results of one study conducted in district Bahawalnagar, Punjab based on the ecology of adult Anopheline species. They found *An. stephensi* and *An. subpictus* as most prevalent species with highest prevalence of mosquitoes in the months of monsoon season (Herrel *et al.* 2004). This shows that monsoon and early post monsoon seasons provide a favorable environment for the breeding of these mosquitoes (Figs 1.3 & 1.4). In support to the present study, almost similar results were also observed in one another study. They also reported higher abundance of Anopheline species in the month of July with the highest abundance of *An. stephensi* (84.3%) as compared to *An. culicifacies* with 2% abundance only (Mukhtar *et al.* 2003).

Conclusion: The result of the present study revealed that the population dynamics of Anopheline mosquitoes vary with the change in geographic boundaries, demographic and climatic conditions.

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