Phlebotomus argentipes Seasonal Patterns in India and Nepal

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ABSTRACT The current control of *Phebotomus argentipes* (Annandale and Brunetti), the vector of *Leishmania donovani* (Laveran and Mesnil), on the Indian subcontinent is base on indoor residual spraying. The efficacy of this method depends, among other factors, on the timing and number of spraying rounds, which depend on the *P. argentipes* seasonality. To describe *P. argentipes'* seasonal patterns, six visceral leishmaniasis (VL) endemic villages, three in Muzaffarpur and three in Sunsari districts in India and Nepal, respectively, were selected based on accessibility and VL incidence. Ten houses per cluster with the highest *P. argentipes* density were monitored monthly for 15–16 mo using Center for Disease Control and Prevention light traps. Minimum and maximum temperature and rainfall data for the months January 2006 through December 2007 were collected from the nearest available weather stations. Backwards stepwise regression was used to generate the minimal adequate model for explaining the monthly variation in *P. argentipes* populations. The seasonality of *P. argentipes* is similar in India and Nepal, with two annual density peaks around May and October. Monthly *P. argentipes* density is positively associated with temperature and negatively associated with rainfall in both study sites. The multivariate climate model explained 57% of the monthly vectorial abundance. Vector control programs against *P. argentipes* (i.e., indoor residual spraying) should take into account the seasonal described here when implementing and monitoring interventions. Monitoring simple meteorological variables (i.e., temperature, rainfall) may allow prediction of VL epidemics on the Indian subcontinent.

KEY WORDS *Phlebotomus argentipes*, visceral leishmaniasis, vector control, sand fly

Visceral leishmaniasis (VL) is a life-threatening vector-borne disease with a fatal outcome if left untreated. The majority of the estimated 500,000 annual VL cases that occur globally are located on the Indian subcontinent, where the disease is especially prevalent in rural communities of India, Nepal, and Bangladesh. In this region, VL is caused by *Leishmania donovani* (Laveran and Mesnil) (Kinetoplastida: Trypanosomatidae) and is transmitted by the sand fly *Phlebotomus argentipes* (Annandale and Brunetti) (Diptera: Psychodidae) (Dinesh et al. 2000). The elimination programs in the region aim to reduce VL by combining active case detection and treatment with widespread use of residual insecticide spraying (IRS) in houses and cattle sheds. However, the rise in the number of VL cases and the number of VL-affected districts in the region in the past decade suggests that the actual strategy is failing to control *L. donovani* transmission (Ostyn et al. 2008). Among other factors (i.e., resistance to first-line drugs) the inadequate implementation of vector control strategies prevails as one of the major causes of this failure in India (Bhattacharya et al. 2006), Nepal (Joshi et al. 2003), and Bangladesh (Mondal et al. 2008). The poor efficacy of vector control programs has been associated with logistical problems, that is, poor quality or inadequate insecticide concentration (Dinesh et al. 2008b), lack of trained personnel, insecticide resistance (Kishore et al. 2004, Ostyn et al. 2008), and inconsistencies in the implementation of control strategies. The latter relates to a poor understanding of *P. argentipes* ecology; indeed the knowledge of the breading sites, the host preference and seasonality of *P. argentipes* are crucial to design an effective vector control program. To maximize the efficacy of IRS, two major factors should be considered (1) the duration of effectiveness of the insecticide used (6 mo or for DDT but only 2 to 5 mo for pyrethroids) and (2) the time of spraying, which depends on the seasonality of the vector (Rozendaal 1997). However, even if previous studies have described the seasonal patterns of *L. donovani* in India (Dinesh et al. 2001) and Nepal (Shrestha 1994), there is no consensus on the best

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strategy and timing to carry out the IRS. Spraying campaign strategies vary in the region, that is, two rounds of DDT in VL-endemic districts in India (Kishore et al. 2006), or focal spraying with alphacypermethrin in villages where VL cases were reported in previous years in Nepal (Joshi et al. 2003). In India, Bihar state sprays two rounds (February–March and May–June), whereas West Bengal state delays the second round until August–October (Kishore et al. 2006). Furthermore, the seasonal patterns of *P. argentipes* depend on ecological and climatic factors that could be monitored to ensure timely implementation of vector control measures and to predict possible VL epidemics in the region (Ghosh et al. 1999). The objective of this study was to provide reliable data on the seasonality of VL vectors in India and Nepal and identify the key meteorological variables responsible for the seasonal patterns in the region.

Materials and Methods

The study areas comprised of two field sites in the Bihar VL focus with three clusters in Muzaffarpur district (in India) and three in Sunsari district (in Nepal). The six clusters were villages or wards with 350–1,500 inhabitants selected based on (1) highest VL incidence rates in the past three years and (2) accessibility all year round. These clusters are a subset of the 26 clusters included in the KALANET trial (ClinicalTrials.gov, CT-2005-015374), which aims to demonstrate the efficacy of long lasting impregnated bednets (LN) to prevent *L. donovani* transmission. The six clusters used in this study did not receive LN (i.e., control clusters in the KALANET trial) but some of the households may have been sprayed as part of the VL control programs in India and Nepal. The detailed method of cluster selection and the impact of LN on *P. argentipes* density have been presented elsewhere (Picado et al. 2010). Twenty five households per cluster were randomly selected in September 2006; sand flies were collected by aspiration for 10 min in up to two rooms per household. In each cluster, the 10 households with the highest number of *P. argentipes* were then monitored with Center for Disease Control and Prevention (CDC) light traps (LT) (Miniature Incandescent Light Trap, model 1012; JW Hock Company, Gainesville, FL) one night per month from 6 p.m. to 6 a.m. for 15 and 16 consecutive months in Nepal and India, respectively, from September 2006.

Informed consent for recruitment into the study was obtained from the head of the households. Ethical clearance from the Indian Council of Medical Research, the ethical committee of B. P. Koirala Institute of Health Sciences in Nepal and the corresponding bodies for each of the institutions involved was obtained.

Sand flies collected in LTs were examined under a binocular dissecting microscope for identification to species and genus for *Phlebotomus* and *Sergentomyia*, respectively. Mosquitoes were recorded to subfamily. All entomological data were double entered into a Microsoft Access database, checked and corrected. The monthly LT collections were summarized using the Williams modified geometric mean (GM) to obtain a single monthly estimate per country (i.e., three clusters combined per country).

The outcome data for the regression analyses were the monthly GM LT total *P. argentipes* collected in the clusters in each country separately, that is, from September 2006 to December 2007 in India and from September 2006 to November 2007 in Nepal. Minimum and maximum temperatures, and rainfall data for the months of January 2006 to December 2007, were collected from the nearest available weather stations, that is, Meteorological station, Tarahara in Nepal and from Rajendra Agricultural University, Pusa in India. All field sites and both stations are in the so called Terai region, a lowland area at the base of the Himalaya range in India and Nepal sharing similar meteorological, ecological, and geological characteristics. Derived climatic variables in the maximal model included lags of 1 to 3 mo for each variable to test whether the density in a given month was influenced not just by the climate in the same month but also by the climate in the previous months which would have affected environmental conditions (i.e., soil moisture) related to survival and development of immature sand flies (Ghosh et al. 1999), thus impacting on current density. Backwards stepwise regression was used to generate the minimal adequate model for explaining the monthly variation in *P. argentipes* indoor density in the two field sites. Only significant variables ($P < 0.05$) were kept in the final model. At this point, the interaction term between country and each of the remaining climatic variables was tested to determine whether the relationship between climate and density varied in the two field sites.

Results and Discussion

In Nepal, out of 450 indoor LT nights, a total of 2,114 *P. argentipes* (46% females) were collected. In India, with a 436 indoor LT nights, 1,049 *P. argentipes* (59% females) were identified.

As shown in Fig. 1a and b, in India *P. argentipes* abundance peaked in October–November 2006 and May–July 2007. In Nepal, *P. argentipes* densities were high in September–November 2006 and May–July 2007. Lowest numbers were detected in January and February 2007 in both countries. These are similar to the results from previous studies in Bihar (Dinesh et al. 2001) and West Bengal states (Ghosh et al. 1999) in India and VL endemic districts in Nepal (Shrestha 1994). However, this is the first time that the same methodology, that is, LTs that are an appropriate tool to determine *P. argentipes* indoor density (Dinesh et al. 2008a), has been applied simultaneously in both countries so regional variations could be studied. The similarity of the patterns observed confirms that Bihar in India and the Terai region in Nepal share similar ecological and climatologic conditions.

Comparison of the observed versus fitted estimates of monthly abundance (Fig. 1a and b) demonstrate

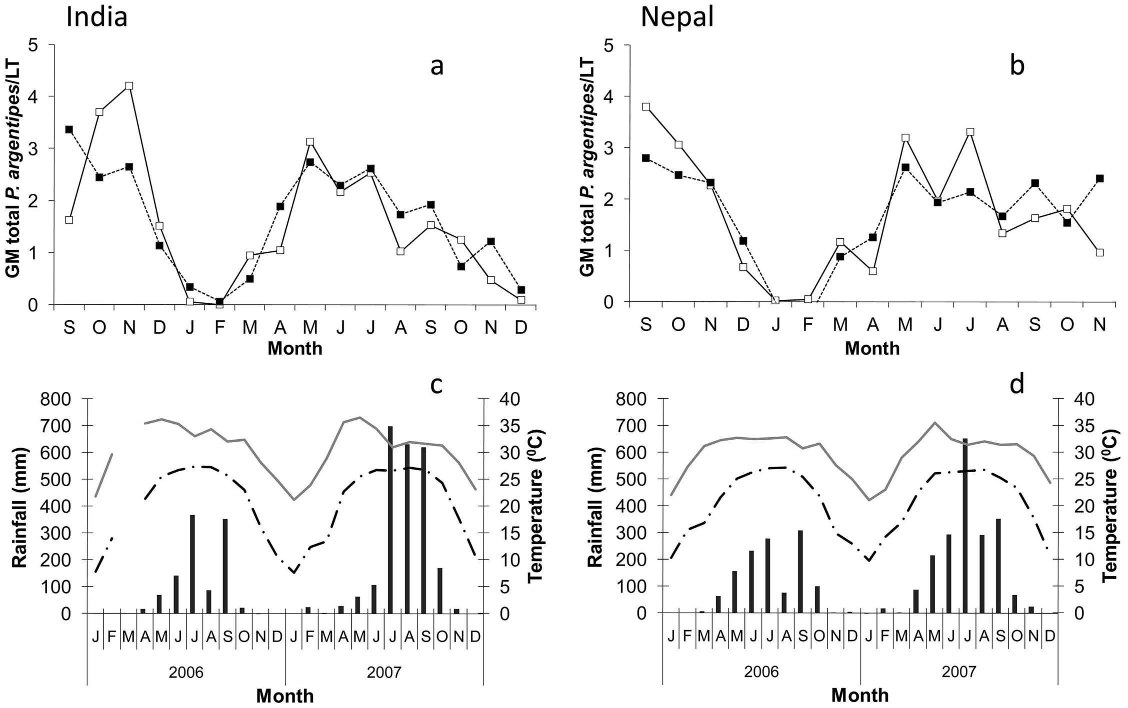

Fig. 1. Observed (solid line) and fitted (dotted line) monthly GM of total *P. argentipes*/LT/house in the three clusters (in each country) from September 2006 to December 2007 in (a) India and (b) Nepal. The fitted line was generated by multivariate regression analysis based on maximum temperature and rainfall. Monthly rainfall (bars) and maximum (solid line) and minimum (dashed lined) temperatures in (c) Muzaffarpur, India and (d) Sunsari, Nepal.

that the seasonal patterns in both countries was well explained by a single regression model incorporating maximum monthly temperature and monthly rainfall (Fig. 1c and d). In both countries, December-February were the coolest months and July and September the rainiest. In the multivariate climate model, the GM monthly abundance (total *P. argentipes*/house LT collection) was positively correlated with the maximum temperature in the same month (coefficient = 0.266/°C, 95% confidence interval [CI] 0.177–0.356, $P < 0.001$) and 3 mo previously (coefficient = 0.151/°C, 95% CI 0.081–0.220, $P < 0.001$); and negatively correlated with total rainfall in the previous month (coefficient = −0.025/cm rain, 95% CI −0.007 through −0.044, $P = 0.009$) and also 3 mo previously (coefficient = −0.019/cm rain, 95% CI −0.001 through −3.65, $P = 0.041$). The model explained 57.3% of the total variance in monthly abundance. After controlling for meteorology, there was no significant difference in abundance between countries, and there were no significant interactions between any of the meteorological variables and country. Hence, a single model with only two meteorological variables was sufficient to explain the differing mean seasonal patterns of *P. argentipes* in the two country field sites. The negative effect of rainfall on *P. argentipes* density was already reported in Bihar (Dinesh et al. 2001) but this is the first time a regression model was used to quantify its effect. Other environmental variables not included in our model (i.e., soil type, pH, moisture, and temperature of soil) contribute to *P. argentipes* abundance (Ghosh et al. 1999) and may have an impact on the sand fly seasonality. The robustness of our climate model for *P. argentipes* should be tested further by comparing model predictions of seasonality across its geographic distribution with further more empirical observations of seasonal patterns, that is, two or three seasonal cycles and more frequent captures.

It was assumed that spraying had a limited effect on seasonality as recent reports indicate that current IRS campaigns are failing to have an impact on *P. argentipes* density in the region (Mondal et al. 2009), in VL endemic villages in Bihar (Dinesh et al. 2008b) and KALANET study clusters in particular (Picado et al. 2010). However, if two rounds of spraying are to be applied as part of the VL control program, insecticide quality should be assured and supervision of spraying improved. The seasonal pattern observed would suggest that February–March and August–September may be the best time to carry out IRS. This should increase the chances that the campaigns have an impact on the disease prevalence (Protopopoff et al. 2007) by spraying the houses in the weeks before the start of the transmission season (Rozendaal 1997). The use of LNs, in combination with IRS, could help overcome vector control problems related to the seasonality and variability of *P. argentipes* density, because LNs provide a sustained insecticidal effect throughout the year (Ostyn et al. 2008).

Although meteorological and predictive models have been used to study some forms of leishmaniasis, that is, cutaneous leishmaniasis in America (Chaves and Pascual 2006), they have rarely been used to predict VL on the Indian subcontinent (Kalluri et al. 2007). Simple climatic variables (i.e., temperature and rainfall) can be used to design predictive models for *P. argentipes* that could help optimize vector control programs in the region.

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