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Research Articles

Application of predictive degree day model for field development of sandfly vectors of visceral leishmaniasis in northwest of Iran

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Abstract

Background & objectives: Temperature plays a significant role in insect's development where a rise in temperature, accelerates the insect's metabolic rates, increases egg production and makes blood feeding more frequent. It also shortens the time period required for the development of pathogens within insects. Visceral leishmaniasis (VL) is one of the most important vector-borne diseases transmitted by different sandfly species. In this study, a phenological model was used to estimate the number of generations, peak activity and temporal variability of sandflies in the main VL foci in northwest Iran.

Methods: Development requirements of different life stages of a *Phlebotomus papatasi* laboratory colony were measured and were subjected to the formula for calculation of accumulated degree day (ADD) for field sandflies using the online soft (UC IPM), using horizontal cut-off method and single triangle model. Sandflies population dynamics was monitored in the field during the seasonal activity in the region and its association with the ADD was tested using SAS software.

Results: Populations of sandflies accommodated well with the amount of accumulated degree days (ADD) in the region. During the seasonal activity, a total of 639 ADD were produced which was enough to support one complete life cycle and growth of the next generation up to late larval instar. Larvae of the second generation hibernate through winter and the first adult population appears in the mid to late June of the next year when they receive at least 182 ADD from the beginning of the spring. The highest population density of sandflies was observed in early August, followed by a rapid decrease in early September, with the adult population disappearing completely in late September. This is the first degree day model related to sandflies in the most important VL foci of Iran.

Interpretation & conclusion: Further studies in various regions with variable climate are recommended in order to better estimate and understand the development time, population dynamics and activities of the vectors which in turn could be used in proper implementation of effective vector control programmes.

Key words Climate – degree day – Iran – leishmaniasis – phlebotomine sandflies – vector

Introduction

Leishmaniasis is caused by a wide range of *Leishmania* species and is transmitted by the phlebotomine sandflies. Currently, more than 500 species of phlebotomine sandflies are recognized, of which about 50 species are known as vectors of *Leishmania* parasites or other human pathogens[1]. Leishmaniasis occurs in about 88 countries, mainly in tropical and subtropical areas with a prevalence of 12 million cases and a global annual incidence of two million cases (all clinical forms). Visceral leishmaniasis (VL) (kala-azar) is a potentially fatal infection with approximately half a million new cases occurring worldwide every year[2]. Most of these cases are attributable to the virulent species *L. donovani*, but *L. infantum* infection (also known as *L. chagasi* in the new world) is more widespread and is endemic in at least 70 countries in Latin America, Africa, Europe and Asia[3–5]. VL caused by *L. infantum* is a zoonosis, and domestic dogs are the principal reservoir of the disease throughout the world.

In Iran, according to records of Ministry of Health, 2056 kala-azar cases were reported during 1997–2006, of which 624 (30.4 %) were from Ardebil Province, northwest of the country[6]. The disease has a complex epidemiological cycle and may have both domestic and wild reservoirs including domestic dogs, foxes, jackals and wolves[7–9]. The principal vectors of VL in the country are the *Phlebotomus* species belonging to the subgenus *Larroussius* (Diptera: Culicidae)[10]. Four sandfly species, namely *P. major, P. kandelakii, P. keshishiani* and *P. perfiliewi transcaucasicus* have been suspected as the vectors of VL in Iran[11–18]. There are currently two main endemic foci of VL caused by *L. infantum*— one in the Ardebil Province in the northwest and the other in Fars Province, south of Iran[7].

The geographical distribution and development of insect vectors are strongly related to the climatic factors such as temperature, rainfall and humidity, among which, temperature plays a prominent role in insect's development. It has a direct correlation with the insects' metabolic rates, egg production, survival of pre-imaginal stages, and adult's longevity and frequency of blood feeding. On the other hand, climatic factors have a direct impact on the development of the pathogens[19–22].

The worldwide increase in prevalence of vector-borne diseases such as VL over the past 20 years, renewed interests in disease surveillance resulting in generating considerable datasets useful for modelling purpose[2,23]. Models based on population dynamics and the environmental parameters make it possible to predict the number of generations per year, the potential ability of population increase, and distribution along temperature gradients[24–25]. Despite the importance of leishmaniasis in Iran, little is known about the impact of climatic factors, such as temperature on sandflies developmental rates and longevity. Information regarding the effects of temperature on the development of sandfly species will undoubtedly improve our knowledge of their population biology and also play a crucial role in planning appropriate vector control programmes. In this study, we investigated the number of generations, peak activity, and temporal variability of sandflies under the field conditions of the main VL focus in northwest of Iran. The thermal requirements of *P. papatasi* under laboratory conditions were used as a model to match and calculate the thermal requirements of the field sandflies.

Material & Methods

Development requirements of P. papatasi under laboratory conditions: Development requirements of *P. papatasi* sandflies were measured on a laboratory colony of the sandfly reared in the insectary of the School of Public Health, Tehran University of Medical Sciences[26]. The days required for the development and the rate of development were measured for each life stage of *P. papatasi*. Development zero (Dz) for each developmental stage of *P. papatasi* determined by Kasap and Alten[27] was used to calculate development requirement, i.e. accumulated degree days (ADD) or the thermal constant (K) of the Iranian lab-strain. The Dz parameter was 11.60, 19.81, 17.63 and 20.25°C for egg, larva, pupa, and pre-oviposition stages, respectively. Based on the Dz and duration of the development, the ADD for each developmental stage of *P. papatasi* lab-strain was calculated using the formula obtained from the web site of the University of California[28]. Simply it was calculated by multiplying the number of days to 28°C (insectarium's temperature) subtracted from minimum threshold requirement.

Study area: Study locations in Germi district (39°1' N latitude and 48°6' E longitude) were in a relatively mountainous region of southern Ardebil province, northwest of Iran. Mean daily maximum temperature in summer is 26°C; and mean daily minimum is 2°C in winter. The mean annual rainfall is more than 350 mm. Entomological survey was carried out in six villages of Hamzeh-khanloo, Kalan-sara, Shah-tapehsee, Sarv-aghaji, Ghasem-kandi, and Hesi-kandi from May to the end of September 2006.

Sandfly collection and identification: Sandflies were captured overnight using Castor oil impregnated A4 papers (sticky traps) which were set in the afternoon and collected in the early morning next day. The collections were made fortnightly for five consecutive months (May–September 2006). At each site, 150 traps were placed, on two consecutive nights in various biotopes, i.e. inside and around human dwellings and animal shelters, close to the vegetation and crevices in walls, and man-made constructs like under bridges. Specimens were counted and stored at 70% ethanol. Before dissection, they were cleaned in Potash 20% and Pouri solution (chloral hydrate/acetic acid), dehydrated and stained with Fuschin 3%. Permanent microscopic slides were made from the remaining body parts of the specimen using Canada balsam[29] before identification according to the shapes of the pharynge, cibarial teeth, spermathecae and male genitalia.

Development requirements of field sandflies: To illustrate the application of the estimation procedure described in Material and Methods, the above mentioned field data were used for the development of sandflies in Germi region. Records of atmospheric temperature (°C) for the whole of 2006 were retrieved from meteorology station of the region. The maximum and minimum temperatures of each day and the development zero[27] of *P. papatasi* were subjected to the formula for calculating ADD for field sandflies using the online software accessible at the website of University of California Agricultural and Natural Resources (Website, UC IPM), using horizontal cut-off method and single triangle model described by Zalom *et al*[30]. Six possible relationships exist between the daily temperature cycle and the upper and lower developmental thresholds. The temperature cycle can be: (i) completely above both thresholds; (ii) completely below both thresholds; (iii) entirely between both thresholds; (iv) intercepted by the lower threshold; (v) intercepted by the upper threshold; or (vi) intercepted by both thresholds. Different equations are required to approximately compute degree days for each case. The relationships between the maximum and minimum temperatures and the developmental thresholds are used to select the proper equation. Equations for each of the six possible cases, using single triangulation are given in Table 1.

Table 1. Formulae for calculating degree days (DD) by the single triangulation method described by Zalom *et al* [30]

Conditions	Equation
$T_{max} > T_U$ & $T_{min} > T_U$	$DD = T_U - T_L$
$T_{max} < T_L$ & $T_{min} < T_L$	$DD = 0$
$T_{max} < T_U$ & $T_{min} > T_L$	$DD = \{6 (T_{max} + T_{min} - 2T_L)\}/12$
$T_{max} < T_U$ & $T_{min} < T_L$	$DD = \{6 (T_{max} - T_L)^2 / (T_{max} - T_{min})\}/12$
$T_{max} > T_U$ & $T_{min} > T_L$	$DD = \{6 (T_{max} + T_{min} - 2 T_L)/12\} - \{6(T_{max} - T_U)^2 / (T_{max} - T_{min})/12\}$
$T_{max} > T_U$ & $T_{min} < T_L$	$DD = \{6 (T_{max} - T_L)^2/(T_{max} - T_{min})\} - \{6(T_{max} - T_U)^2/(T_{max} - T_{min})/12\}$

T_{max}: Maximum daily temperature; T_{min}: Minimum daily temperature; T_U: Upper temperature threshold (i.e. 35°C); T_L (=Dz): Lower temperature threshold.

Statistical analysis: Logistic regression was performed to test the association between temperature or ADD and the population density of sandflies using SAS software[31]. The resulting DD values or daily mean temperature are plotted versus the population density of the field sandflies captured in the corresponding time.

Results

Development requirements: Calculated degree-day requirements for each life stage of *P. papatasi* under laboratory conditions are given in Table 2. Excluding the pre-oviposition period, which showed wide range of 28.75 to 109.25 DD, the lowest requirement was for the eggs (86.4 DD) and the highest requirement was for the IV larval stages (154.75 DD). For pupa to adult, it was 142.29 DD. The accumulated egg to adult degree days requirement was 383.44 DD and when that of the pre-oviposition period was added, it amounted to 412.19–492.69 DD. The average rate of development (1/d, where d is the time required in days by each developmental stage) for egg to adult emergence was 0.02%.

Table 2. Details of development days, rate of development, development zero (Dz), and accumulated degree day (ADD) for *P. papatasi* sandflies under laboratory conditions (26±1 and 80% RH). Maximum developmental threshold used in this experiment was 35°C and the minimum developmental thresholds (Dz) is taken from Kasap and Alten[27]

Stage	Development days	Rate of development	Dz	ADD
Egg	6	0.170	11.60	086.40
Larva	25	0.040	19.81	154.75
Pupa	17	0.060	17.63	142.29
Egg to adult	48	0.020	11.6 for egg 19.81 for larva 17.63 for pupa	383.44
Pre-oviposition	5–19	0.050	20.25	28.75–109.25
Egg to egg	53–67	0.015–0.017	11.6–20.25	412.19–492.69

Sandfy fauna and entomological survey: During the seasonal activity of the sandflies, the population is generally high and more than 20,000 specimens were collected from the study area. Three thousand specimens were randomly selected (1400 females and 1600 males) for morphological identification of the species. A total of 13 different sandfly species including 10 *Phlebotomus* and three *Sergentomyia* were identified in the study area. *P. perfiliewi transcaucasicus*, a known vector of visceral leishmaniasis (61%) was the most frequent and *P. mongolensis* (0.32%) was the least abundant sandfly species. The species composition of other known sandflies were *P. papatasi* (6.1%), *P. (adlerius)* spp (5.9%) (including *P. brevis, P. halepensis* and *P. longiductus*), *P. sergenti* (5.3%), *P. kandelakii* (3.1%), *P. alexandri* (1.5%) and *P. tobbi* (1.5%).

Population dynamics: The proportion of sandflies density was significantly ($p < 0.05$) associated with the corresponding temperature or ADD. The relatively high R^2 (R^2=0.5) value of linear regression of number of sandflies and DD or temperature indicate that the relationship between these two variables is strong. Population dynamics of sandflies in the region and the impact of environmental temperature and corresponding ADD on population fluctuations are shown in Fig. 1. Field investigation on the population density of sandflies in the region showed that the first adult population occurred in mid-June and the highest population density was observed in the second week of August, followed by a sharp decrease in the third week of September, with the adult population disappearing completely in mid-September. Based on the results, it can be said that in Germi region, late, presumably the III or the IV instar larvae of sandflies hibernate during the cold seasons (autumn and winter). The ADD from early January to mid-June was approximately 182 DD when the minimum threshold temperature (Dz) equal to 18°C was taken into calculation. The diapaused IV instar larvae received 39 ADD during the early spring (May to mid-June) necessary to complete their development to pupae. They then received enough accumulated degree-days (142 DD) to convert to adults in

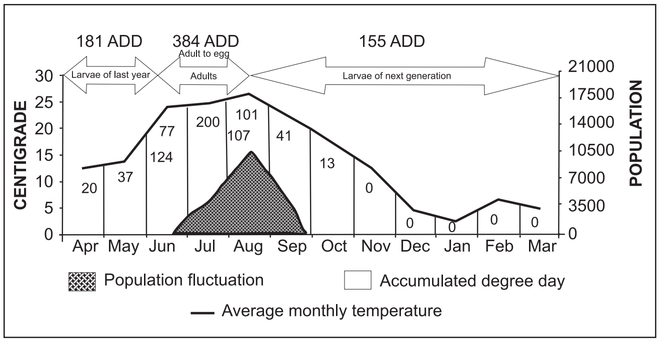

Fig. 1: Correlation of environmental temperature, accumulated degree days (ADD) and population fluctuation of sandflies in Germi, Ardebil, north-western Iran in 2006. ADD of each month is shown on the corresponding column (ADD of August was calculated for two separate 15-days). ADD produced for different life stages are shown on the top of the arrows. Specific development zero (Dz) used for eggs, larvae, pupae, and pre-oviposition stages were 11.60, 19.81, 17.63 and 20.25°C respectively.

mid-June when average temperature was 24°C. This is in concordance with the ADD (182 DD) necessary for development of *P. papatasi* larvae and pupae under the laboratory conditions. Thereafter, the density of population increased, they mated and laid eggs, larvae and pupae completed their development, and the new generation adults appeared in early August and peaked in the second week of August, since the average minimum temperature in June, July and August (21–24°C) is sufficient for completion of their development. During mid-June to the second week of August, circa 454 DD was produced in the region when the minimum threshold temperature was considered 20.25, 11.6, and 18°C for development of pre-oviposition, egg, and larvae-pupae respectively. This condition is adequate for the adults of the first generation of sandflies to emerge in the region. This measurement is within the range of the ADD calculated for the development of a complete life cycle (egg to egg) of *P. papatasi* (412.19 – 492.69) under laboratory conditions.

The first generation females are ready to oviposit in the mid to late August. The larvae of the second generation are produced subsequently since the average temperatures (26°C) in the late August and early September is sufficient for eggs to hatch (considering Dz equal to 11.60°C and K is 86.4 DD). However, average temperature (17.5°C) in September cannot provide the ADD needed for successful development of all four larval stages where Dz is 19.81°C and it requires at least 154.75 DD. Calculation of ADD from mid-August to the end of September was about 185 DD when the average temperature was 17.5°C which is less than minimum threshold temperature (Dz = 19.81°C) for the completion of larval development. In response to the decline in temperature as well as daytime, these late larvae (presumably the III and IV instars) become dormant and because of the lack of the second generation adults, the population density starts to decrease through September and finally the adult populations disappear in the late September in Germi. This means that the sandflies in the study area are monovoltine, i.e. produce only one generation per year.

Discussion

We demonstrated that the predictive degree day model is a useful tool for estimating the development, population density, and number of generations of sandflies in the field. This model provides valuable information about temporal and spatial distribution of vectors of VL in the region. The relative contribution of climatic factors in explaining temporal variability in disease incidences, to a large extent, determines the practical utility of a degree day model. The epidemiological data reported by health authorities in the region showed that the VL cases occurred in September onwards[6]. This is in agreement with the population dynamics of the sandfly vectors particularly *P. perfeliewi transcaucasicus* which was the dominant (61%) sandfly species in the region. We detected a close association between the population dynamics of *P. perfeliewi transcaucasicus* and their infection to *L. infantum/donovani*, with a significant increase in *L. infantum/donovani* prevalence in the sandfly populations from June to August 2006[13]. The peak of *P. perfeliewi transcaucasicus* activity in August is responsible for most of the transmission from reservoirs (dogs) to humans. As temporal dynamics of the vector populations are crucial for the maintenance of the pathogens, the high activity of *P. perfeliewi transcaucasicus* in August[13–16] would provide a much higher probability of VL transmission than from early season sandflies.

For infectious diseases where the pathogen replicates outside the final host (e.g. in a vector), climate factors have a direct impact on the development of the pathogen. Most viruses, bacteria and parasites do not replicate below a certain temperature threshold, for example 18°C for the malaria parasite *Plasmodium falciparum* and 20°C for the Japanese encephalitis virus[20,32]. Studies of Rioux *et al*[33] showed that the optimum temperature for the development of *L. infantum* in sandflies was circa 25°C. The average temperature in August was 26°C which is the warmest month in the region. Higher temperature not only accelerates the development of sandfly vectors but also shortens the development time of *Leishmania* parasite in the vectors[33–34]. A study on the influence of temperature on the life cycle of *L. infantum* in *P. ariasi* by Rioux *et al*[33] showed that raising the temperature significantly increased the overall proportion of infected sandflies, speeded up the multiplication of promastigotes in the midgut, controlled the movement of parasites forward into the thoracic midgut (from 15°C), and encouraged the attachment of the flagellates to the wall of the stomodaeal valve (from 20°C)[33].

In this study we attempted to use developmental requirements such as development zero (Dz) and degree days required for the development of *P. papatasi* in the laboratory for sandfly species in nature including *P. papatasi* and *P. perfeliewi transcaucasicus*. Although this may result in some inaccuracy in our estimation model, the *P. papatasi* laboratory model adequately described the development of both *P. papatasi* and *P. perfeliewi transcaucasicus* under the field conditions. The accuracy of the model will be improved when breeding of *P. perfeliewi transcaucasicus* in the laboratory becomes possible. In nature, in addition to temperature, other factors such as substrate availability (nutritional deficiencies, effects of fluctuating temperature), enzyme availability (hormonal effects on growth, effects of fluctuating temperature), and approximations and assumptions in calculating the minimum and maximum threshold temperature for development could influence the model and should be considered in further studies[19,21].

The available datasets which were the results of intensive research and disease surveillance following the surge in the prevalence of VL in the world in the last 20 years are useful for modelling purposes[35]. Specific knowledge of vector phenology is the centre piece of the effective vector control campaigns. Models based on population dynamics and the environmental parameters facilitate the prediction of the number of generations per year, the potential ability of population increase, and distribution along temperature gradients[24–25]. The findings of this study better identify the factors affecting vector abundance and dynamics, thereby contributing to our understanding of the dynamics of Leishmania infections in the region and could help health authorities in intelligent engagement in the disease and vector control programmes.

Conclusion

Specific knowledge of vector phenology is essential in effective vector control programmes. These kinds of studies are crucial for better understanding the factors affecting vector abundance and dynamics, as well as the dynamics of *Leishmania* infections in the region and could help health authorities in proper management and adequate deployment of the disease and vector control measures.

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