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Seasonal trends of sand fly abundance and *Leishmania* infection: The case of Bologna province, Italy (2016–2023)

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ABSTRACT

Leishmaniasis is a vector-borne disease, caused by *Leishmania* parasites, transmitted by phlebotomine sand flies. Recently, shifts in its geographic distribution have been observed across Europe, including Italy, with climate changes believed to influence sand fly proliferation, altering disease transmission. Using meteorological, entomological, and visceral leishmaniasis (VL) data (2016–2023) from the province of Bologna, Italy, this study aimed at examining the relationships between meteorological factors and sand fly abundance, *Leishmania* infection in sand flies, and human VL cases. Entomological data showed the peak of sand fly abundance in July–August, followed by a peak of the infection rate one month later. Sand fly abundance resulted negatively correlated with spring cumulative precipitation ($r = -0.93$, 95 % CI: −1.00, −0.62) and mean relative humidity ($r = -0.81$, 95 % CI: −1.00, −0.29) while a positive correlation was observed between yearly sand fly abundance and number of VL cases in the following year ($r = 0.82$, 95 % CI: 0.34, 1.00). A negative correlation was also found between cumulative precipitation from March to June and number of VL cases in the following year ($r = -0.71$, 95 % CI: −1.00, −0.07). These findings suggest that reduced precipitation may contribute to increased sand fly abundance within the same season and potentially lead to higher number of notified human VL cases in the following year. Our study highlights the importance of meteorological factors as potential predictors of leishmaniasis. Considering these findings, we propose that public health measures, such as information campaigns and the use of repellents, could be strengthened during drier years, provided that our results can be replicated in other regions with different environmental contexts to ensure broader applicability.

List of abbreviations

VL	Visceral leishmaniasis

1. Introduction

Leishmania parasites are the causative agents of leishmaniasis, a family of infectious diseases affecting both humans and animals [1]. The pathogens are transmitted through bites of infected female Phlebotomine sand flies. In humans, infection by different *Leishmania* species can lead to three primary clinical forms: Cutaneous (CL), Mucosal (ML), and Visceral Leishmaniasis (VL). Of these, VL has the longest incubation period, ranging from 3 to 8 months, and presents the most severe, potentially life-threatening, clinical outcomes [2].

Countries in Southern Europe, including Italy, Spain, France, and Greece, are endemic for the L. *infantum* parasite, the main cause of VL and CL cases in the region [1]. In recent decades, VL and CL have spread northward from their endemic region in southern Europe to areas previously considered unsuitable for leishmaniasis transmission [3–5].

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In Italy, northern regions such as Piedmont, Emilia-Romagna, and Veneto - historically non-endemic for leishmaniasis - have reported an increase in the presence of vectors and in human cases [6–10]. This shifted distribution may be influenced by changes in climatic conditions [7,11], since the life cycle of sand flies is highly dependent on factors such as temperature, humidity, and precipitation [12,13].

Temperature has a strong influence on sand fly development, longevity, and fecundity [13–15], while humidity and precipitation, are essential for egg survival, despite the absence of an aquatic stage in their life cycle [12,13,16,17]. However, the impact of precipitation on sand fly abundance is unclear and appears to vary by geographical area and species [18–21].

Although many studies have reported the effects of meteorological conditions on sand fly occurrence, relatively few have focused on the relationships between meteorological factors before the activity period of sand flies and subsequent sand fly abundance and incidence of human leishmaniasis. However, meteorological conditions before the sand fly activity period are likely to play an important role in shaping sand fly habitats and influencing their life cycle [12,13].

To address this gap, our study analyzes long-term meteorological, entomological, and human VL data (2016–2023) from a northern Italian area that has experienced an increasing number of VL cases in recent years [6]. We hypothesize that climate variables, particularly temperature, and precipitation in the months before sand fly activity period, influence vector population dynamics and subsequent disease transmission. Our objective is to examine the relationships between meteorological factors before the sand fly activity period and the seasonal abundance of sand flies, *Leishmania* infection in sand flies, and human VL cases.

2. Materials and methods

2.1. Sand fly abundance and Leishmania infection rate

Sand flies were collected between 2016 and 2023 at a single site in Monteveglio, Valsamoggia municipality, Bologna province (44°28′48.6"N, 11°05′02.9″E), at an altitude of 196 m. The site is a wild area around an isolated, abandoned house, surrounded by the hilly landscape featuring cultivated fields, vineyards, hedgerows, and woodlands on hilltops and steep slopes.

Depending on the different seasons of monitoring, a variable number of CDC-light traps, and CO_2-baited traps (CAA model) were placed for one night, from sunset to sunrise, at the sampling site, every two weeks from June to October, to collect sand flies. The captured sand flies were refrigerated, transported to the laboratory and killed by freezing. Males and females were then separated, and males were morphologically identified after clarification, following the standard taxonomy keys [22]. Number of sand flies were recorded separately for traps used, sex, and collection dates. Then, the female samples were pooled within pool sizes of 50–100 individuals and prepared for Real-Time PCR test for the presence of *Leishmania* spp. [23] The number of tested pools and of positive pools were recorded according to collection dates.

For each sampling month (July–September) and year (2016–2023), we defined the sand fly monthly abundance as the highest number of captured sand flies/trap/night across all active traps in the month. This choice allowed us to control the large variability in the number of captured sand flies, with some traps capturing few sand flies, while others capturing substantially large numbers of sand flies. The yearly abundance was calculated by averaging the monthly abundance within the same year.

Leishmania infection rate in pooled samples was assessed by Maximum Likelihood Estimation (MLE) of the proportion of infected sand flies. With this method, the infection rate is defined as the proportion of infected sand flies that maximises the likelihood that a given number of pools of a given size are positive for the pathogens, assuming a binomial distribution of infected individuals in a positive pool [24–26]. For each sampling month (July–September) and year (2016–2023), monthly infection rates were computed using the total number of positive pools, the total number of tested pools of each month, and the average pool size. The yearly infection rates were calculated using the cumulative total of positive pools and tested pools from July to September of each year. This analysis was performed using the PooledInfRate package in R [26,27].

2.2. Meteorological factors

Daily mean temperature, daily mean relative humidity, and daily cumulative precipitation data at the sampling site were obtained from the ERG5 dataset Version 2, provided by ARPAE (Regional Environment Agency) [28]. To characterize weather conditions before the activity period of sand flies, the average of daily mean temperature and daily mean relative humidity between March and June were calculated every year. We also computed cumulative precipitation for the same period each year.

2.3. Human Leishmaniasis cases

Data on human visceral Leishmaniasis cases in the Bologna province from 2016 to 2023, identified through the national surveillance system, were obtained from the Regional Health Authority of Emilia-Romagna. Only human VL cases were included in our analysis.

2.4. Analyses

The relationships among meteorological factors, sand fly abundance, *Leishmania* infection in sand flies, and human VL cases, represented in Fig. 1, are likely to be complex, entailing both direct and indirect effects and possible interactions. In this paper, we adopted a simplified approach and attempted to disentangle the various associations by analysing the pairwise linear relationships by means of the Spearman's correlation coefficients between i) meteorological parameters and yearly sandflies abundance, ii) meteorological parameters and yearly *Leishmania* infection rate, iii) yearly abundance and yearly *Leishmania* infection rate, iv) meteorological parameters and number of human VL cases in the following year, and v) yearly sand flies abundance and number of human VL cases in the following year.

The reason for choosing a 1-year lag between meteorological parameters and VL human cases and between vector abundance and VL human cases is due to duration of the sand fly activity period and the prolonged incubation period of VL, which typically ranges from 3 to 8

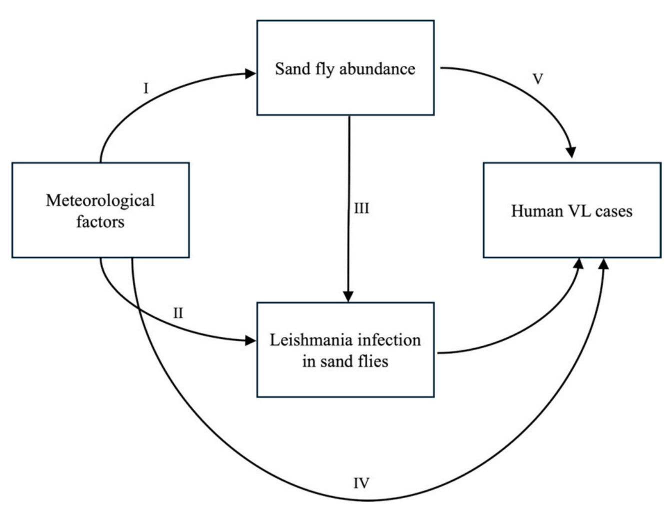

Fig. 1. Relationship among meteorological factors, sand fly abundance, *Leishmania* infection in sand flies, and in humans.

months [2].

To study further the association between sand fly abundance and *Leishmania* positivity in sand flies, we adopted a previously used approach [29], comparing probabilities of *Leishmania* positivity in sandflies from binomial distribution models, built from simulated and observed datasets, where simulations provided a distribution of infection rates that was independent of the abundance of sand flies (more details are provided in supplementary material S8).

All the analyses were carried out using R software Version 2024.04.1 + 748 (Posit Software, PBC, 2024).

3. Results

3.1. Descriptive results

In the 8-year period of observation, from 2016 to 2023, traps were active for 66 days of sampling. Yearly abundance fluctuated over time, with the highest capture recorded in July 2021 (n = 9505). That year also saw the highest average maximum capture number (n = 5378), while the lowest was in 2023 (n = 83) (Table 1). Despite these fluctuations, the seasonal trend remained consistent, peaking in July or August. Similarly, the *Leishmania* infection rate in sand flies varied by year. The highest infection rate was observed in 2017, with 26.6 infected sand flies/1000, while the lowest occurred in 2022, at 2.5/1000 (Table 2). The seasonal trend showed relatively low infection rate in June and July, increasing to achieve a peak in August and September, approximately one month after the peak abundance (Supplementary S6).

Monthly mean temperature, monthly mean relative humidity, and cumulative precipitations for the period of observation (2016–2023) are shown in supplementary material S1-S3. During the observation period, the average mean temperature and mean relative humidity across March and June ranged from 15.2 °C to 16.9 °C and 60.2 % to 70.8 %, respectively. Cumulative precipitation for this period each year ranged from 254 to 483 mm (Fig. 2).

3.2. Impact of meteorological factors on sand fly abundance and Leishmania infection rates

Spearman's correlation test showed no correlation between the yearly mean temperature from March to June and yearly sand fly abundance (r = 0.12, 95 % CI: −0.78, 0.97), or yearly infection rate (r = − 0.17, 95 % CI: −0.97, 0.63) in the period of July to September. However, a strong negative correlation was observed between both the yearly mean relative humidity and yearly sand fly abundance (r = −0.81, 95 % CI: −1.00, −0.29), and between cumulative precipitation and yearly sand fly abundance (r = −0.93, 95 % CI: −1.00, −0.62) (Fig. 2, Supplementary S4). No correlation was found between weather variables and the yearly infection rate. (Fig. 3, Supplementary S5).

Table 1
Sand fly monthly abundance and yearly abundance.

Year	Jun	Jul	Aug	Sep	Oct	Avg
2016	–	2728	1084	1050	–	1620
2017	2952	4548	5600	549	–	3565
2018	88	1139	1419	169	4	909
2019	30	500	1455	133	–	696
2020	100	2295	464	247	–	1002
2021	2174	9505	4731	1899	18	5378
2022	200	4432	1900	1068	–	2466
2023	–	123	48	79	53	83

Avg: average of the highest number of sand flies captured monthly between July and September.

Table 2
Monthly and yearly number of pools tested, number of *Leishmania* positive pools, and infection rates in 2016–2023.

	Infection Rate (Positive pools/ Number of tested pools)					
Year	Jun	Jul	Aug	Sep	Oct	Yearly total
2016	–	1.1 (1/12)	69.2 (14/14)	19.3 (6/7)	–	11.5 (21/33)
2017	1.8 (5/59)	2.1 (1/10)	39.5 (26/30)	45.0 (10/10)	–	26.6 (42/109)
2018	0.0 0/2)	1.1 (2/46)	6.3 (7/32)	0.0 (0/3)	–	2.9 (9/83)
2019	0.0 (0/1)	4.2 (3/17)	4.0 (18/104)	11.0 (1/4)	–	4.1 (22/126)
2020	0.0 (0/1)	0.8 (1/27)	9.7 (5/14)	0.0 (0/4)	–	3.1 (6/46)
2021	0.0 (0/14)	0.0 (0/15)	11.9 (11/16)	38.8 (12/13)	NA (1/1)	7.6 (24/59)
2022	–	3.8 (11/63)	1.1 (2/40)	0.0 (0/9)	–	2.5 (13/112)
2023	–	0.0 (0/2)	49.3 (2/2)	0.0 (0/2)	NA (2/2)	12.5 (4/8)

3.3. Sand fly abundance and Leishmania infection rates

No correlation was observed between yearly sand fly abundance and yearly infection rates (r = 0.07, 95 % CI: −0.85, 0.75) (Supplementary S7).

To test further this result, we compared the probability of infection from models fitted to simulated and observed datasets. Model estimates were similar (Supplementary S9), with almost complete overlap of the probability of positive pools estimated using observed and synthetic data, the latter assuming independence between infection rate and abundance. The results support that the abundance of sand flies is not associated with an increase in their infection rate.

3.4. Association of meteorological factors and sand fly abundance with VL human cases

Bologna province reported 83 cases of VL between 2017 and 2024. The highest number of incident cases was observed in 2018 (N = 18). A strong positive correlation was found between yearly sand fly abundance and the number of human VL cases observed in the following year (r = 0.82, 95 % CI: 0.34, 1.00). Analogously, cumulative precipitation from March to June each year was negatively correlated with the number of human VL cases in the following year (r = −0.71, 95 % CI: −0.97, −0.24). (Fig. 4, Supplementary S10-S11).

4. Discussion

In our study, every year, the abundance of sand flies peaked between July and August, followed by a peak of *Leishmania* infectivity approximately one month later. We observed strong negative correlations between cumulative precipitation from March to June and sand fly abundance from July to September during the same year, and between cumulative precipitation from March to June in a given year with human VL cases recorded in the Bologna province in the following year. There was no correlation between yearly sand fly abundance and *Leishmania* infection rates in sand flies in the same year, but yearly abundance positively correlated with the number of human VL cases recorded during the following year.

Our findings are consistent with a previous study in the same area, which reported the highest sand fly abundance in a year with the lowest cumulative precipitation during the surveillance season [30]. This suggests that heavy rainfall during spring months may create unfavorable conditions for breeding and survival of sand flies in the surveyed site, likely due to waterlogging and flooding in breeding sites or lower ambient temperatures associated with rainfall, which can hinder the

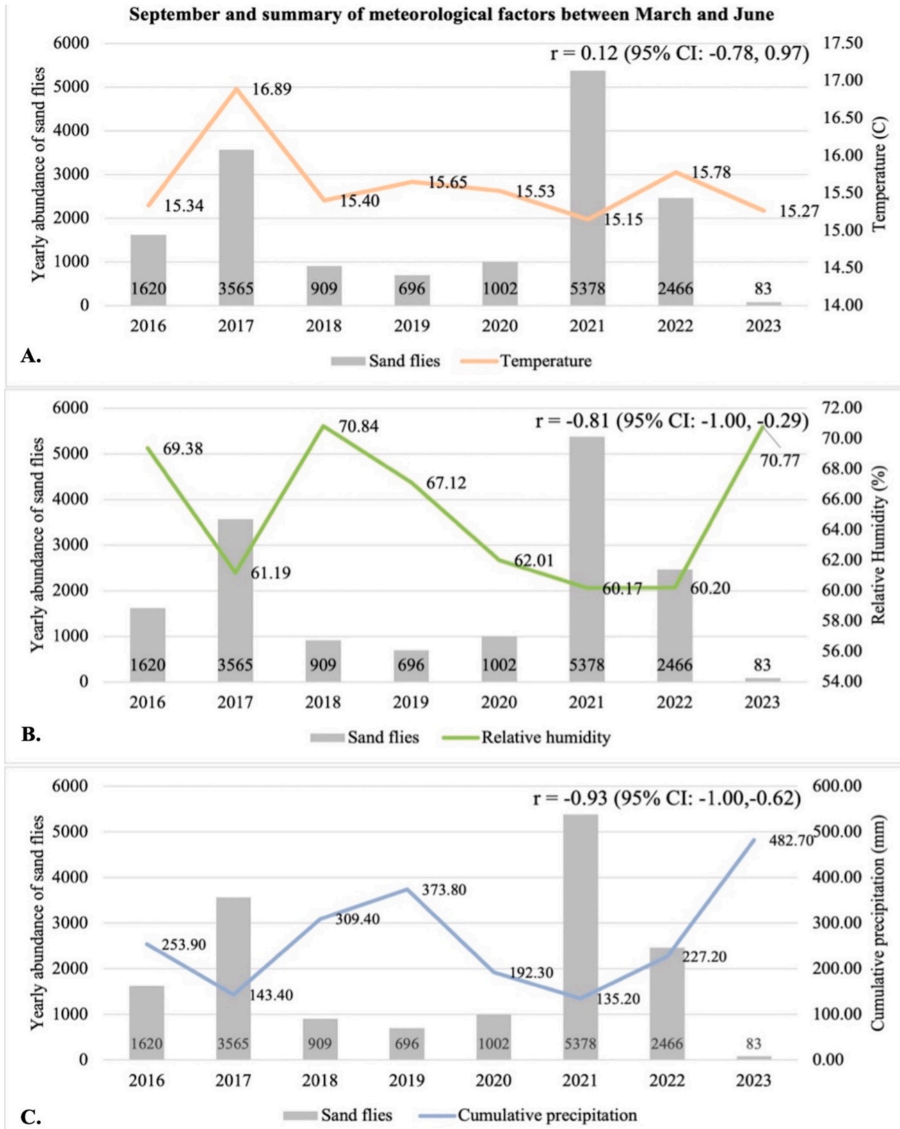

Fig. 2. Yearly abundance and summary of meteorological factors from March to June. (A) Average of daily mean temperature from March to June and yearly abundance, (B) Average of daily mean relative humidity from March to June and yearly abundance, (C) Cumulative precipitation from March to June and yearly abundance.

development of sand fly life cycle [11,31,32]. Additionally, this effect of heavy rainfall may outweigh the influence of temperature on sand fly abundance, which could explain the lack of correlation between temperature and abundance observed in our study. The absence of this correlation may also be attributed to an exposure window (March to June) which is too large to capture the potential short-term association between temperature and sand fly abundance [33–35].

We found a positive correlation between yearly sand fly abundance in the site and the number of human VL cases recorded in the following year in the Bologna province. This relationship aligns with the observed negative correlation between cumulative precipitation from March–June and the number of human VL cases recorded in the following year. Similar patterns were reported in the area, with an unusually dry summer in 1971 before a VL outbreak in the Bologna province, where 60 cases were recorded between 1971 and 1973 [36]. Our interpretation is that low cumulative precipitation may lead to an increase in sand fly abundance thereby elevating the risk of disease transmission and subsequently contributing to a rise in human leishmaniasis incidence after an extended time lag [37].

Importantly, our results indicate that dry conditions before sand fly activity period could serve as an early warning signal for potential VL outbreaks. Moreover, sand fly abundance appears to be a more reliable predictor of human VL incidence than *Leishmania* infection rates in sand flies [38–41]. This emphasizes the need for surveillance and control efforts to prioritize monitoring sand fly population as a key indicator of disease risk, rather than infection rate in vectors.

The one-year lag between abundance of vectors and human VL case occurrence may reflect the complex transmission dynamics of *Leishmania* [12,13,38,42–44], whereby an increase in sand fly populations in a given year may lead to higher transmission rates and, consequently, a rise in human cases in the following year. This delayed effect is likely influenced by the incubation period of the disease and its diagnosis, and the time it takes for infected sand flies to transmit *Leishmania* to human hosts [2,13,42,45]. These findings highlight the need for proactive disease surveillance in response to changing environmental conditions, also considering that climate change projections predict decreased precipitation in the Mediterranean region, particularly during spring [46–49].

In our study, some limitations should be acknowledged. First, although the study revealed strong correlations between meteorological factors, vector abundance, and *Leishmania* infection, these findings

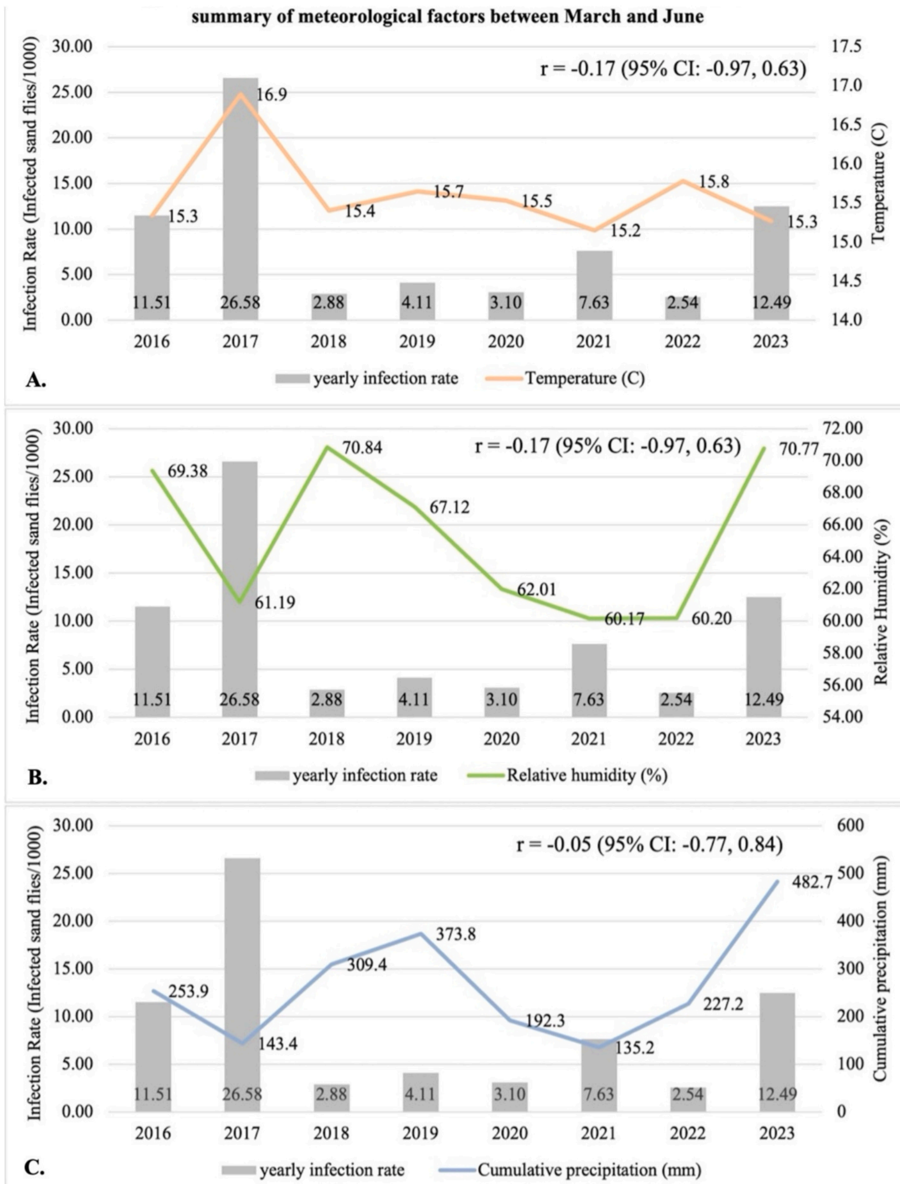

Fig. 3. Yearly *Leishmania* infection rate in sand flies and summary of meteorological factors from March to June. (A) Average of daily mean temperature from March to June and yearly *Leishmania* infection rate, (B) Average of daily mean relative humidity from March to June and yearly *Leishmania* infection rate, (C) Cumulative precipitation from March to June and yearly *Leishmania* infection rate.

cannot be interpreted as evidence of causal relationships or as indicative of time-lag exposure-outcome associations. Second, *Leishmania* infection in sand flies was tested using Real-Time PCR with a pooling method, which is fast, effective and suitable for routine surveillance [50]. However, tests were performed on insect pools, and the number of infected sand flies within a positive pool was not available. Lastly, the collection of sand flies at a single site may not adequately represent abundance at the province scale, where Leishmaniasis cases were reported. It also limits our ability to assess variations in the meteorological impact on the vector population and parasite circulation across different environmental settings.

Future research should replicate similar studies in other regions with different weather conditions and environmental settings to broaden applicability of our findings. Including factors such as land use type could provide insights into how meteorological factors link to sand fly abundance and human VL cases. Moreover, statistical modeling could help adjust for potential confounders, improving our understanding of the relationships between meteorological factors and sand fly abundance and human VL cases. This approach could also identify key determinants of sand fly abundance and human VL cases, informing public health strategies for more effective disease surveillance and prevention.

5. Conclusions

Our findings underline the importance of meteorological factors, particularly precipitation and humidity, in shaping sand fly population dynamics. The observed associations between sand fly abundance and human VL cases underscore the relevance of sand fly monitoring as a predictive tool for anticipating disease outbreaks. These results highlight the need for an integrated approach to VL control that considers both environmental and biological factors in the transmission cycle, including climate-driven changes in sand fly populations and the timing of possible control interventions to mitigate the public health impact of VL in endemic regions.

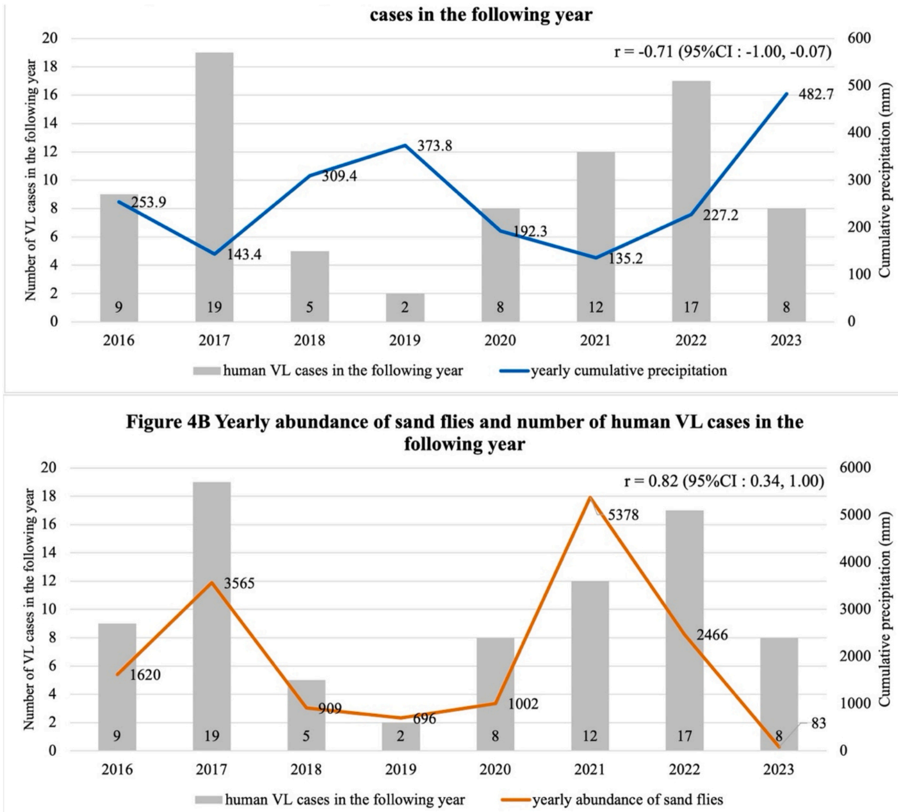

Fig. 4. (A) Cumulative precipitation from March to June in the year (indicated on the horizontal axis (blue line) and number of human VL cases in the following year (bar chart), (B) Yearly abundance of sand flies in the year (indicated on the horizontal axis (red line) and number of human VL cases in the following year (bar chart). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CRediT authorship contribution statement

Juthathip Khongpetch: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis. **Giovenale Moirano:** Writing – review & editing, Software, Methodology, Formal analysis, Conceptualization. **Annalisa Grisendi:** Writing – review & editing, Data curation. **Mara Scremin:** Writing – review & editing, Data curation. **Giovanna Mattei:** Writing – review & editing, Data curation. **Arianna Puggioli:** Writing – review & editing, Data curation. **Paola Angelini:** Writing – review & editing, Data curation. **Giulio Matteo:** Writing – review & editing, Data curation. **Michele Dottori:** Writing – review & editing, Data curation. **Milena Maule:** Writing – original draft, Supervision, Methodology. **Mattia Calzolari:** Writing – review & editing, Supervision, Resources, Data curation, Conceptualization.

Author agreement statement

We, the authors, declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the Corresponding Author is the sole contact for the editorial process and is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

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Declaration of competing interest

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.

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Appendix A. Supplementary data

https://docs.google.com/document/d/1uS7mVcbaaeyZ0wucaW4aFC0aFClSp4eb2WbFifGU_cA/edit?usp=sharing

Data availability

Data are available on request.

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