

## Article

# Spatial Analysis of Different Clinical Forms of Leishmaniasis in Honduras from 2009 to 2016

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## Abstract

Leishmaniasis is a tropical and subtropical disease caused by protozoans of the genus *Leishmania*, primarily transmitted to humans through the bite of sandflies. This neglected disease poses a serious global health challenge due to its spectrum of clinical manifestations, which can lead to potentially fatal outcomes. In Honduras, four clinical forms of leishmaniasis are present: ulcerative cutaneous leishmaniasis (UCL), mucosal leishmaniasis (ML), non-ulcerated cutaneous leishmaniasis (NUCL), and visceral leishmaniasis (VL). This study aims to identify spatial patterns of these four clinical forms of the disease in Honduras, utilizing epidemiological data from 2009 to 2016. Geographic Information System (GIS) analysis was employed for spatial assessment. Moran's I was used to evaluate the data and reveal patterns, while Hot Spot Analysis identified statistically significant spatial clusters of high and low values. For UCL and NUCL, all Global Moran's I *p*-values were below 0.001 throughout the study period. For VL, *p*-values were under 0.001 in 2010, 2013, and 2016. For ML, *p*-values were below 0.05 in 2009, 2011, 2014, and 2015. In conclusion, our findings demonstrate geographical segregation among the different clinical forms of leishmaniasis.

**Keywords:** spatial epidemiology; hot-spot analysis; incidence of leishmaniasis; temporal analysis

## 1. Introduction

Leishmaniasis is a tropical and subtropical disease caused by protozoans of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae), primarily transmitted to humans through

sandfly bites. In 2023, 272,098 new cases of cutaneous leishmaniasis and 11,922 of visceral leishmaniasis were reported in 200 countries worldwide [1]. This neglected disease represents a serious world health problem, particularly in Latin America, due to its broad spectrum of clinical manifestations that can result in fatal outcomes [2,3]. In Honduras, four clinical forms of leishmaniasis are recognized: ulcerative cutaneous leishmaniasis (UCL), non-ulcerative cutaneous leishmaniasis (NUCL), mucosal leishmaniasis (ML), and visceral leishmaniasis (VL), each determined by clinical characteristics, their geographic distribution, parasite species, and vectors involved [4–9].

The first case of VL reported in Honduras occurred in 1977 in the southern region of the country [10]. Fourteen years later, in 1988, a variant of cutaneous leishmaniasis caused by *Leishmania (L.) infantum chagasi* [6] was identified in the same area. In contrast, the UCL and ML forms are primarily found along the Caribbean coast [11]. In Honduras, three species of *Leishmania* have been reported: *Leishmania (L.) infantum chagasi* [8,12,13], which is associated with both VL and NUCL, and is predominantly distributed in the southern part of the country; *Leishmania (V.) braziliensis* [12], which is associated with UCL and ML, and is found in the northeastern region; and *Leishmania (V.) panamensis* [12], which is associated only with UCL and circulates in the northwestern part of the country.

Despite the prevalence of the disease in this region, the spatial and temporal distribution of leishmaniasis throughout Honduras has not yet been studied. Therefore, this study aims to identify spatial patterns of the different clinical forms of leishmaniasis reported in Honduras, based on epidemiological data from 2009 to 2016. The data were provided by the National Health Secretary's Chagas and *Leishmania* National Program and analyzed at the sub-regional government level (local municipalities). We also evaluated the characteristics of the patterns (clustered, dispersed, or random) and identified spatial clusters within the country.

## 2. Materials and Methods

### 2.1. Study Area and Data

Honduras is located in northern Central America (15°11'60" N 86°14'30.9" W), and has a population of 9,880,507 across 111,890 km<sup>2</sup> [14]. The climate is tropical, with an estimated average annual rainfall of 1500 mm. The yearly average maximum and minimum temperatures are 35 °C and 19 °C, respectively. Honduras is divided into 18 regional governments known as departments and 298 municipalities (Figure 1).

Leishmaniasis is a reportable disease under the surveillance of the Honduran Ministry of Public Health. All leishmaniasis cases registered at the municipality level for the period 2009–2016 were provided by the National Health Secretary's Chagas and *Leishmania* National Program (<http://www.salud.gob.hn/site/index.php>, accessed on 20 October 2023). The database does not include names or personal information of patients.

The incidence data were categorized as cutaneous leishmaniasis (UCL and NUCL), ML, and VL. Cases of UCL and NUCL are confirmed through microscopic analysis of the biopsy or cutaneous scraping, which usually reveals the parasite in its amastigote stage. VL is confirmed based on clinical symptomatology or via an rK39-positive quick test that utilizes bone marrow material for microscopic analysis of the parasite in its amastigote stage, according to the "Resolution DGM-M04:2016, Integral Approach Manual of Leishmaniasis in Honduras" [15]. The database includes the confirmed cases of the four clinical forms of leishmaniasis in Honduras during 2009 and 2016, along with age group classifications.



**Figure 1.** Map of Honduras showing the geographical position in Central America.

## 2.2. Leishmaniasis Morbidity Analysis

The population data by year for calculating the incidence rate of leishmaniasis (2009–2016) were obtained from the Honduran National Institute of Statistics (<https://ine.gob.hn/bases-de-datos/>, accessed on 20 October 2023). The geographic layers for the country outline and administrative subdivisions were obtained from the Center for Spatial Sciences at the University of California. These layers contained a single-class vector format (polygons). The disease incidence rate was calculated from the total number of cases per 10,000 inhabitants in each municipality, divided by the total number of people at risk within the municipality.

## 2.3. Spatial Analysis

The information on the four clinical forms of leishmaniasis reported in Honduras was mapped and analyzed using a geographic information system (ArcGIS Pro 2.5.1) [16]. Incidence maps were created to highlight the distribution of leishmaniasis at the municipality level, and Global Moran's I was used to evaluate whether the geographic pattern of incidence was clustered, dispersed, or random, based on the calculation of spatial autocorrelation of the data. Finally, a Hot Spot Analysis based on the Getis–Ord  $G_i^*$  statistic was employed to identify statistically significant spatial clusters of high and low incidence values. The Getis–Ord  $G_i^*$  statistic uses the null hypothesis of randomization as the basis for the test of statistical significance.

### 3. Results

#### 3.1. Distribution of Cases and Incidence

Between 2009 and 2016, 15,345 cases of leishmaniasis were confirmed in 112 municipalities across 14 (77.7%) of the 18 Honduran departments. The department with the highest number of cases was Olancho, with 4064 cases (26.5%), with an incidence rate of 75.2, whilst Copan was the department with the lowest number of cases (7 cases, 0.04%, incidence rate 0.7, as was Lempira (Table 1). UCL was the most prevalent form of the disease in the country, with 9167 cases (59.73%), followed by NUCL with 6055 cases (39.45%), ML with 92 cases (0.59%), and VL with 31 cases (0.20%) (Figure 2). The distribution of incidence remained stable from 2011 to 2015, followed by an increase in 2016, with 2652 cases (an incidence rate of 3.86 per 10,000 inhabitants) (Figure 3).

**Table 1.** Epidemiological overview of leishmaniasis in Honduras, during the period 2009–2016.

| Department        | Total Cases | Incidence Rate | UCL Cases | Incidence Rate | CL % | NUCL Cases | Incidence Rate * | NUCL % | ML Cases | Incidence Rate * | ML % | VL Cases | Incidence Rate * | VL % |
|-------------------|-------------|----------------|-----------|----------------|------|------------|------------------|--------|----------|------------------|------|----------|------------------|------|
| Yoro              | 291         | 5.4            | 291       | 5.4            | 3.2  | 0          | 0.0              | 0.0    | 0        | 0.0              | 0.0  | 0        | 0.0              | 0.0  |
| Gracias a Dios    | 18          | 2.3            | 18        | 2.3            | 0.2  | 0          | 0.0              | 0.0    | 0        | 0.0              | 0.0  | 0        | 0.0              | 0.0  |
| Colón             | 1315        | 40.1           | 1300      | 39.7           | 14.2 | 7          | 0.2              | 0.1    | 8        | 0.2              | 8.7  | 0        | 0.0              | 0.0  |
| Lempira           | 10          | 0.7            | 5         | 0.4            | 0.1  | 5          | 0.4              | 0.1    | 0        | 0.0              | 0.0  | 0        | 0.0              | 0.0  |
| Santa Bárbara     | 1074        | 26.2           | 1066      | 26.0           | 11.6 | 8          | 0.2              | 0.1    | 0        | 0.0              | 0.0  | 0        | 0.0              | 0.0  |
| La Paz            | 9           | 16.0           | 0         | 0.0            | 0.0  | 9          | 16.0             | 0.1    | 0        | 0.0              | 0.0  | 0        | 0.0              | 0.0  |
| Atlántida         | 329         | 7.3            | 302       | 6.7            | 3.3  | 8          | 0.2              | 0.1    | 19       | 0.4              | 20.7 | 0        | 0.0              | 0.0  |
| Comayagua         | 24          | 1.0            | 7         | 0.3            | 0.1  | 17         | 0.7              | 0.3    | 0        | 0.0              | 0.0  | 0        | 0.0              | 0.0  |
| Cortés            | 1545        | 10.1           | 1508      | 9.9            | 16.5 | 36         | 0.2              | 0.6    | 1        | 0.0              | 1.1  | 0        | 0.0              | 0.0  |
| Olancho           | 4064        | 75.2           | 3831      | 70.9           | 41.8 | 184        | 3.4              | 3.0    | 49       | 0.9              | 53.3 | 0        | 0.0              | 0.0  |
| El Paraíso        | 1106        | 23.6           | 647       | 13.8           | 7.1  | 458        | 9.8              | 7.6    | 1        | 0.0              | 1.1  | 0        | 0.0              | 0.0  |
| Francisco Morazán | 742         | 5.3            | 133       | 0.9            | 1.5  | 607        | 4.3              | 10.0   | 0        | 0.0              | 0.0  | 2        | 0.0              | 6.5  |
| Valle             | 1283        | 71.4           | 16        | 0.9            | 0.2  | 1266       | 70.4             | 20.9   | 0        | 0.0              | 0.0  | 1        | 0.1              | 3.2  |
| Choluteca         | 3528        | 79.8           | 36        | 0.8            | 0.4  | 3450       | 78.0             | 57.0   | 14       | 0.3              | 15.2 | 28       | 0.6              | 90.3 |
| Copán             | 7           | 0.7            | 7         | 0.7            | 0.1  | 0          | 0.0              | 0.0    | 0        | 0.0              | 0.0  | 0        | 0.0              | 0.0  |
|                   | 15345       |                | 9167      |                |      | 6055       |                  |        | 92       |                  |      | 31       |                  |      |

\* Rate per 10,000 inhabitants, CL (cutaneous leishmaniasis), NUCL (non-ulcerated cutaneous leishmaniasis), ML (mucosal leishmaniasis) and VL (visceral leishmaniasis).

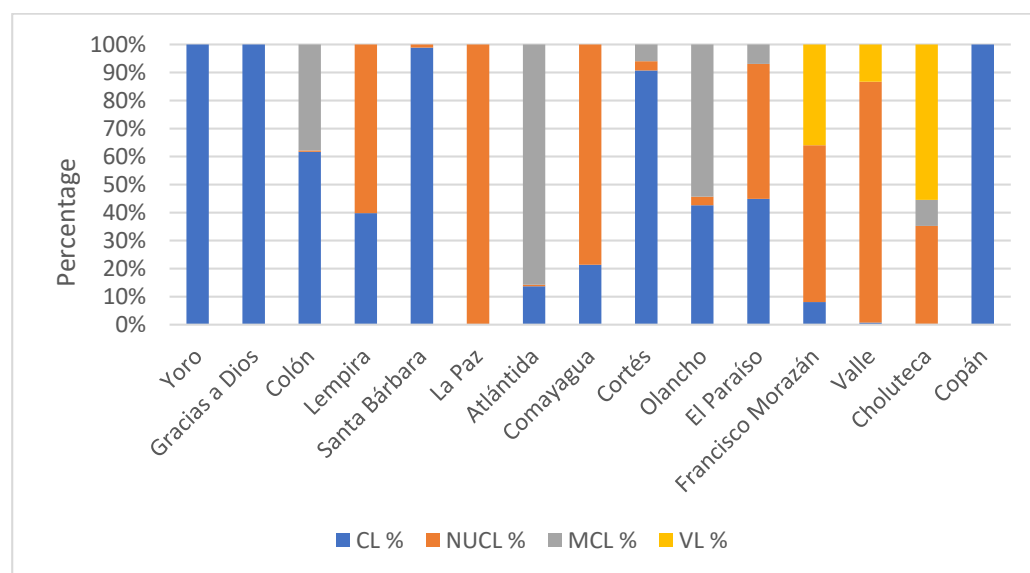


**Figure 2.** Distribution of leishmaniasis cases by clinical form and health region registered in Honduras during the period 2009–2016. CL (cutaneous leishmaniasis), NUCL (non-ulcerated cutaneous leishmaniasis), ML (mucocutaneous leishmaniasis), and VL (visceral leishmaniasis).

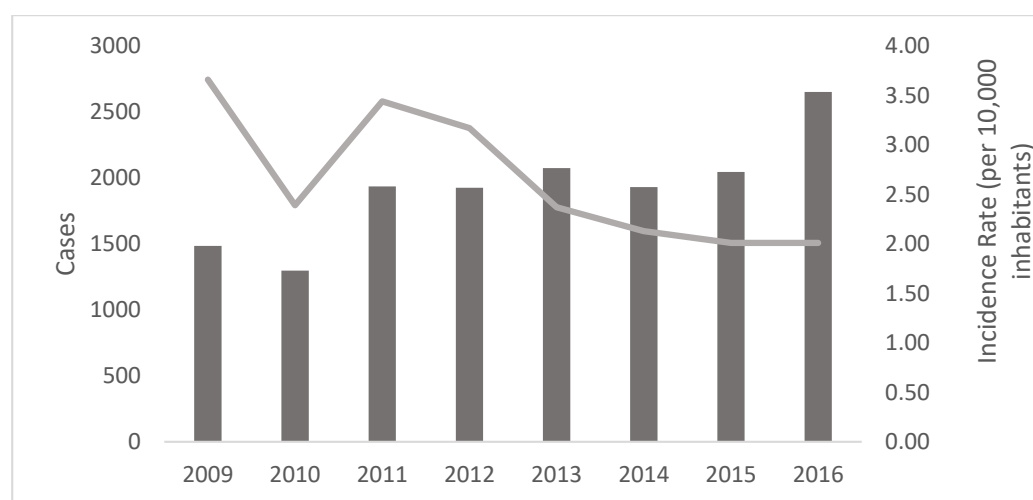


**Figure 3.** Distribution of cases (bars) and incidence rate (line) of leishmaniasis registered in Honduras during the period 2009–2016.

### 3.2. Age and Gender

The gender incidence could not be analyzed for the entire period, as the only available information began in 2013. A significant statistical difference was found between female and male cases ( $p < 0.0001$ ), with 3288 cases (37.78%) in females and 5415 cases (52.48%) in males. The incidence rates between both groups also showed a significant statistical difference ( $p = 0.006$ ) (Table 2).

**Table 2.** Cases and incidence rate of leishmaniasis according to gender during the period 2013–2016 in Honduras.

| Year  | General |       |  | Females |       |                  | Males |      |                  |
|-------|---------|-------|--|---------|-------|------------------|-------|------|------------------|
|       | Cases   | %     |  | Cases   | %     | Incidence Rate * | Cases | %    | Incidence Rate * |
| 2013  | 2075    | 23.8  |  | 697     | 21.19 | 1.16             | 1316  | 24.3 | 2.19             |
| 2014  | 1931    | 22.2  |  | 771     | 23.34 | 1.24             | 1175  | 21.7 | 1.89             |
| 2015  | 2045    | 23.5  |  | 881     | 26.80 | 1.29             | 1197  | 22.1 | 1.76             |
| 2016  | 2652    | 30.5  |  | 943     | 28.67 | 1.37             | 1727  | 31.9 | 2.52             |
| Total | 8703    | 100.0 |  | 3288    | 100   |                  | 5415  | 100  |                  |

\* Rates per 100,000 inhabitants;  $\chi^2 = 246.86$ ,  $p \leq 0.0001$ .

The age group with the highest number of cases was 20–49 years, with 3336 cases (38.33%), followed by 10–19 years with 2884 cases (33.13%). The lowest number of cases was in the age group of <5 years, with 724 cases (8.31%). A significant statistical difference was found among the age groups ( $p = 0.0001$ ) (Table 3).

**Table 3.** Cases and incidence rate of leishmaniasis by age group during the period 2013–2016 in Honduras.

| Year  | <5 Years |                  | 5–9 Years |                  | 10–19 Years |                  | 20–49 Years |                  | >50 Years |                  |
|-------|----------|------------------|-----------|------------------|-------------|------------------|-------------|------------------|-----------|------------------|
|       | Cases    | Incidence Rate * | Cases     | Incidence Rate * | Cases       | Incidence Rate * | Cases       | Incidence Rate * | Cases     | Incidence Rate * |
| 2013  | 152      | 1.78             | 252       | 2.95             | 727         | 8.50             | 756         | 8.84             | 202       | 2.36             |
| 2014  | 129      | 1.53             | 190       | 2.25             | 650         | 7.71             | 733         | 8.69             | 199       | 2.36             |
| 2015  | 182      | 2.12             | 165       | 1.92             | 668         | 7.79             | 820         | 9.56             | 225       | 2.62             |
| 2016  | 261      | 2.99             | 282       | 3.23             | 839         | 9.62             | 1027        | 11.78            | 244       | 2.80             |
| Total | 724      |                  | 889       |                  | 2884        |                  | 3336        |                  | 870       |                  |

\* Rates per 100,000 inhabitants; F-value = 11.37,  $p = 0.0001$ .

### 3.3. Hot Spot and Cluster Analysis

#### 3.3.1. Incidence Distribution

The incidence rates of UCL, ML, NUCL, and VL varied between 0 and 75, 0 and 35, 0 and 135, and 0 and 2 cases per 10,000 inhabitants, respectively.

In the three years analyzed (Figure 4), UCL presented an incidence of 35 or greater in the departments of Olancho, Colón, El Paraíso, and Francisco Morazán. ML was only recorded in 2012, showing a rate of  $\geq 35$  in one of its municipalities, Choluteca. The NUCL had a rate of  $\geq 65$  in the Choluteca department, affecting up to three municipalities (2009 and 2012), and in one municipality of Valle in 2016, located in the southern part of the country. VL exhibited a similarly low incidence rate across all three years, also being concentrated in the southern region.

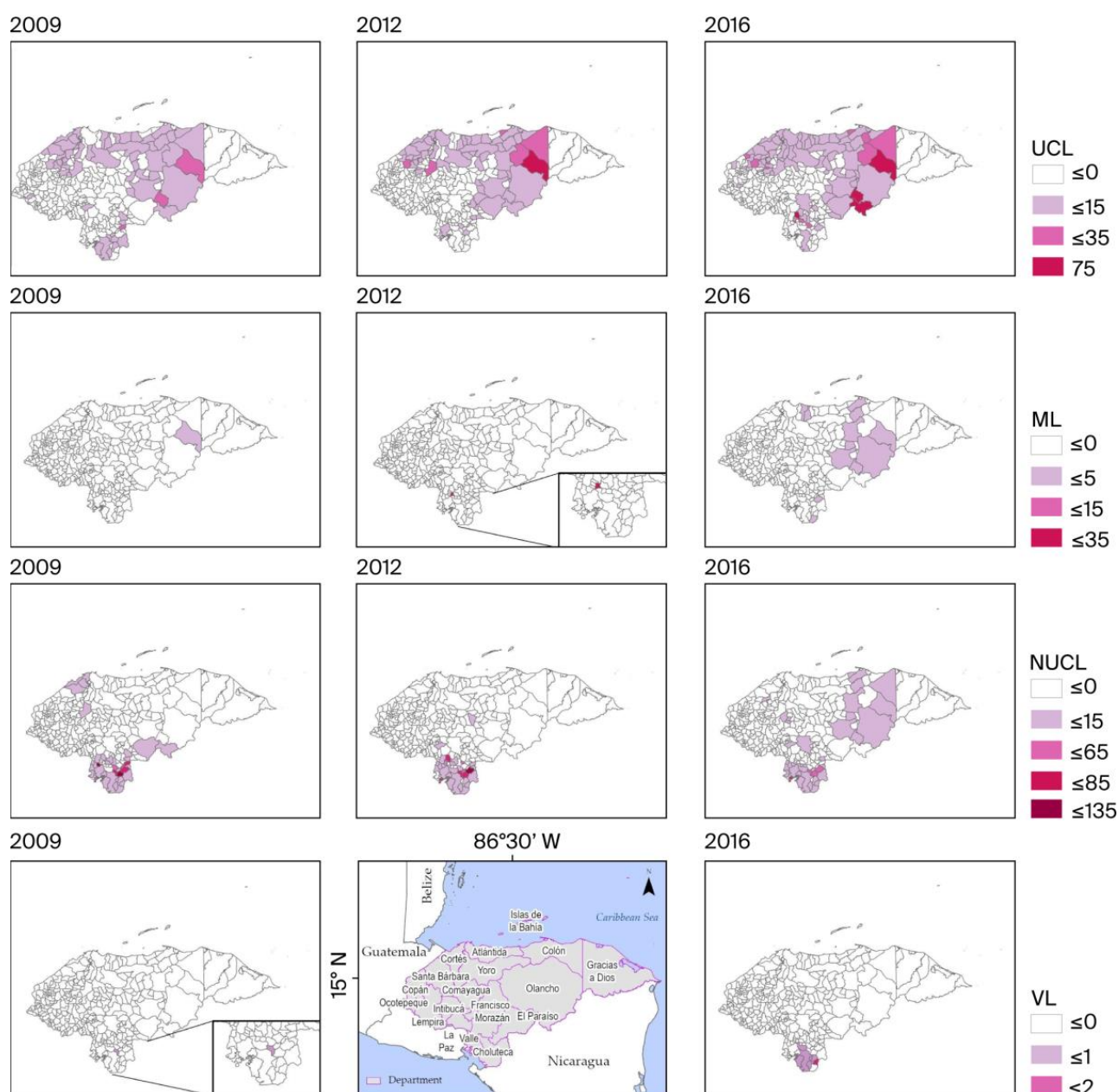


**Figure 4.** Incidence rate distribution. Note: No available data for VL incidence in 2012.

### 3.3.2. Global Spatial Autocorrelation (Global Moran's I)

Table 4 displays the results of the global spatial autocorrelation test for Global Moran's I of all clinical forms of leishmaniasis. For UCL and NUCL, spatial autocorrelation was significant ( $p$ -value  $< 0.001$  throughout the whole period), as was the case for VL in 2010, 2013, and 2016. There is less than a 1% likelihood that this cluster pattern could result from random chance. For ML, significant autocorrelation occurred in 2009, 2011, 2014, and 2015 ( $p$ -value  $< 0.05$ ).

**Table 4.** Global Spatial Autocorrelation (Global Moran's I).

| Year | Moran's I | UCL<br>z-Score | p-Value  | Moran's I | ML<br>z-Score | p-Value  | Moran's I | NUCL<br>z-Score | p-Value  | Moran's I | VL<br>z-Score | p-Value  |
|------|-----------|----------------|----------|-----------|---------------|----------|-----------|-----------------|----------|-----------|---------------|----------|
| 2009 | 0.039573  | 3.907069       | 0.000093 | 0.010298  | 2.492981      | 0.012668 | 0.146927  | 13.480736       | 0.000000 | −0.003354 | 0.004182      | 0.996663 |
| 2010 | 0.063343  | 6.475721       | 0.000000 | 0.002619  | 1.883818      | 0.059590 | 0.097058  | 9.975942        | 0.000000 | 0.049209  | 5.04188       | 0.000000 |
| 2011 | 0.031314  | 3.869622       | 0.000109 | 0.019649  | 2.671906      | 0.007542 | 0.113616  | 11.585693       | 0.000000 | 0.012615  | 1.895551      | 0.058019 |
| 2012 | 0.044129  | 5.395738       | 0.000000 | −0.005884 | −0.790557     | 0.429203 | 0.146254  | 13.397129       | 0.000000 | **        | **            | **       |
| 2013 | 0.047576  | 4.885245       | 0.000001 | 0.005041  | 0.977825      | 0.328161 | 0.144689  | 14.469943       | 0.000000 | 0.057306  | 6.799102      | 0.000000 |
| 2014 | 0.074792  | 6.841419       | 0.000000 | 0.034964  | 3.805863      | 0.000141 | 0.084995  | 8.677335        | 0.000000 | −0.000563 | 0.882399      | 0.377561 |
| 2015 | 0.070127  | 6.379846       | 0.000000 | 0.015346  | 2.201847      | 0.027676 | 0.144997  | 13.876317       | 0.000000 | 0.003423  | 0.68698       | 0.492096 |
| 2016 | 0.072236  | 6.315367       | 0.000000 | 0.014101  | 1.624236      | 0.104325 | 0.078689  | 8.549363        | 0.000000 | 0.118458  | 11.910415     | 0.000000 |

UCL: ulcerative cutaneous leishmaniasis, ML: mucocutaneous leishmaniasis, NUCL: non-ulcerative cutaneous leishmaniasis, VL: visceral leishmaniasis. \*\* No cases of visceral leishmaniasis were registered in 2012.

### 3.3.3. Hot Spot Analysis (Getis–Ord Gi\*)

The identification of hot spots for each clinical form of leishmaniasis was based on the Getis–Ord Gi\* statistic using a fixed distance band in ArcGIS software. The Gi\*z-score ranged from 1.67 to 17.23, while the GiPValues ranged from 0 to 0.09 for the entire period, indicating a clustered pattern with 95% confidence.

According to Table 5, the results show that the clinical forms NUCL\_09 to NUCL\_16 exhibit Z-scores between 5.8 and 11.9 with  $p < 0.01$ , indicating a high spatial concentration of elevated values, interpreted as highly significant hot spots. This behavior suggests the presence of geographic areas where the cases or values associated with these forms occur more frequently than would be expected by chance. In contrast, the forms UCL\_09 to UCL\_16 mostly display Z-scores near zero and non-significant  $p$ -values ( $p > 0.05$ ), indicating a random spatial distribution, except for UCL\_10, which shows moderate clustering ( $Z = 2.269$ ;  $p < 0.05$ ). Similarly, the ML and VL forms exhibit mixed results: some could not be evaluated due to a lack of spatial variation (Error 000906), while others, such as VL\_10, VL\_13, and VL\_16, show highly significant spatial clustering ( $Z > 4$ ;  $p < 0.01$ ).

The Getis–Ord Gi\* maps for UCL and ML reflect critical points mainly located in the eastern part of the country, specifically in the departments of Colón and Olancho for most of the entire period (Gi\*z-score  $> 1.6$ ,  $p$ -values  $< 0.05$ ), except for the San José municipality in the Choluteca department in 2012, located in the southern region. Since 2016, both clinical forms of leishmaniasis have shown a hot spot trend in some municipalities in the southern departments of Honduras, such as Choluteca (ML) and Francisco Morazán (UCL). Conversely, NUCL and VL exhibited a statistically significant hot-spot spatial pattern only in the southern part of the country across all observations during the period. In contrast, there was a noticeable decrease in the probability of clustering of hot spots or cold spots for all four clinical forms toward the western part of the country (Figure 5).

**Table 5.** Global Hot-Cold Pattern Analysis Using Getis-Ord General G.

| Clinical Form Code | Observed General G | Expected General G | Z-Score | p-Value              | Statistical Significance | Spatial Pattern Interpretation                                     |
|--------------------|--------------------|--------------------|---------|----------------------|--------------------------|--------------------------------------------------------------------|
| NUCL_09            | 0.00003            | 0.000005           | 10.64   | 0                    | $p < 0.01$               | High spatial clustering of elevated values (significant hot spots) |
| NUCL_10            | 0.000029           | 0.000005           | 8.068   | 0                    | $p < 0.01$               | High spatial clustering of elevated values (significant hot spots) |
| NUCL_11            | 0.000034           | 0.000005           | 10.141  | 0                    | $p < 0.01$               | High spatial clustering of elevated values (significant hot spots) |
| NUCL_12            | 0.000029           | 0.000005           | 10.524  | 0                    | $p < 0.01$               | High spatial clustering of elevated values (significant hot spots) |
| NUCL_13            | 0.000032           | 0.000005           | 11.933  | 0                    | $p < 0.01$               | High spatial clustering of elevated values (significant hot spots) |
| NUCL_14            | 0.000024           | 0.000005           | 6.757   | 0                    | $p < 0.01$               | High spatial clustering of elevated values (significant hot spots) |
| NUCL_15            | 0.000032           | 0.000005           | 11.023  | 0                    | $p < 0.01$               | High spatial clustering of elevated values (significant hot spots) |
| NUCL_16            | 0.00002            | 0.000005           | 5.866   | 0                    | $p < 0.01$               | High spatial clustering of elevated values (significant hot spots) |
| UCL_09             | 0.000005           | 0.000005           | −0.376  | 0.70684              | Not significant          | Random spatial distribution, no evidence of clustering             |
| UCL_10             | 0.00001            | 0.000005           | 2.269   | 0.0233               | $p < 0.05$               | Moderate clustering of elevated values (weak hot spots)            |
| UCL_11             | 0.000005           | 0.000005           | 0.008   | 0.99325              | Not significant          | Random spatial distribution                                        |
| UCL_12             | 0.000004           | 0.000005           | −0.777  | 0.4369               | Not significant          | Random spatial distribution                                        |
| UCL_13             | 0.000005           | 0.000005           | −0.084  | 0.93281              | Not significant          | Random spatial distribution                                        |
| UCL_14             | 0.000007           | 0.000005           | 0.989   | 0.32225              | Not significant          | Random spatial distribution                                        |
| UCL_15             | 0.000005           | 0.000005           | 0.172   | 0.86308              | Not significant          | Random spatial distribution                                        |
| UCL_16             | 0.000006           | 0.000005           | 0.812   | 0.41657              | Not significant          | Random spatial distribution                                        |
| ML_09              | —                  | —                  | —       | —                    | Error 000906             | Analysis failed (identical values, zero variance)                  |
| ML_10              | —                  | —                  | —       | —                    | Error 000906             | Analysis failed (identical values, zero variance)                  |
| ML_11              | 0.000011           | 0.000005           | 0.717   | 0.4736               | Not significant          | Random spatial distribution                                        |
| ML_12              | —                  | —                  | —       | —                    | Error 000906             | Analysis failed (identical values, zero variance)                  |
| ML_13              | —                  | —                  | —       | —                    | Error 000906             | Analysis failed (identical values, zero variance)                  |
| ML_14              | 0.000012           | 0.000005           | 1.415   | 0.15694              | Not significant          | Random spatial distribution                                        |
| ML_15              | 0.000003           | 0.000005           | −0.491  | 0.62374              | Not significant          | Random spatial distribution                                        |
| ML_16              | 0.000009           | 0.000005           | −0.666  | 0.50528              | Not significant          | Random spatial distribution                                        |
| LV_09              | —                  | —                  | —       | —                    | Error 000906             | Analysis failed (identical values, zero variance)                  |
| LV_10              | 0.000039           | 0.000005           | 4.038   | $5.4 \times 10^{-5}$ | $p < 0.01$               | Significant clustering of elevated values (hot spots)              |
| LV_11              | 0.000023           | 0.000005           | 1.984   | 0.04723              | $p < 0.05$               | Moderate clustering of elevated values (weak hot spots)            |
| LV_12              | —                  | —                  | —       | —                    | Error 000906             | Analysis failed (identical values, zero variance)                  |
| LV_13              | 0.000065           | 0.000005           | 5.804   | 0                    | $p < 0.01$               | High spatial clustering of elevated values (significant hot spots) |
| LV_14              | —                  | —                  | —       | —                    | Error 000906             | Analysis failed (identical values, zero variance)                  |
| LV_15              | —                  | —                  | —       | —                    | Error 000906             | Analysis failed (identical values, zero variance)                  |
| LV_16              | 0.00005            | 0.000005           | 9.853   | 0                    | $p < 0.01$               | High spatial clustering of elevated values (significant hot spots) |

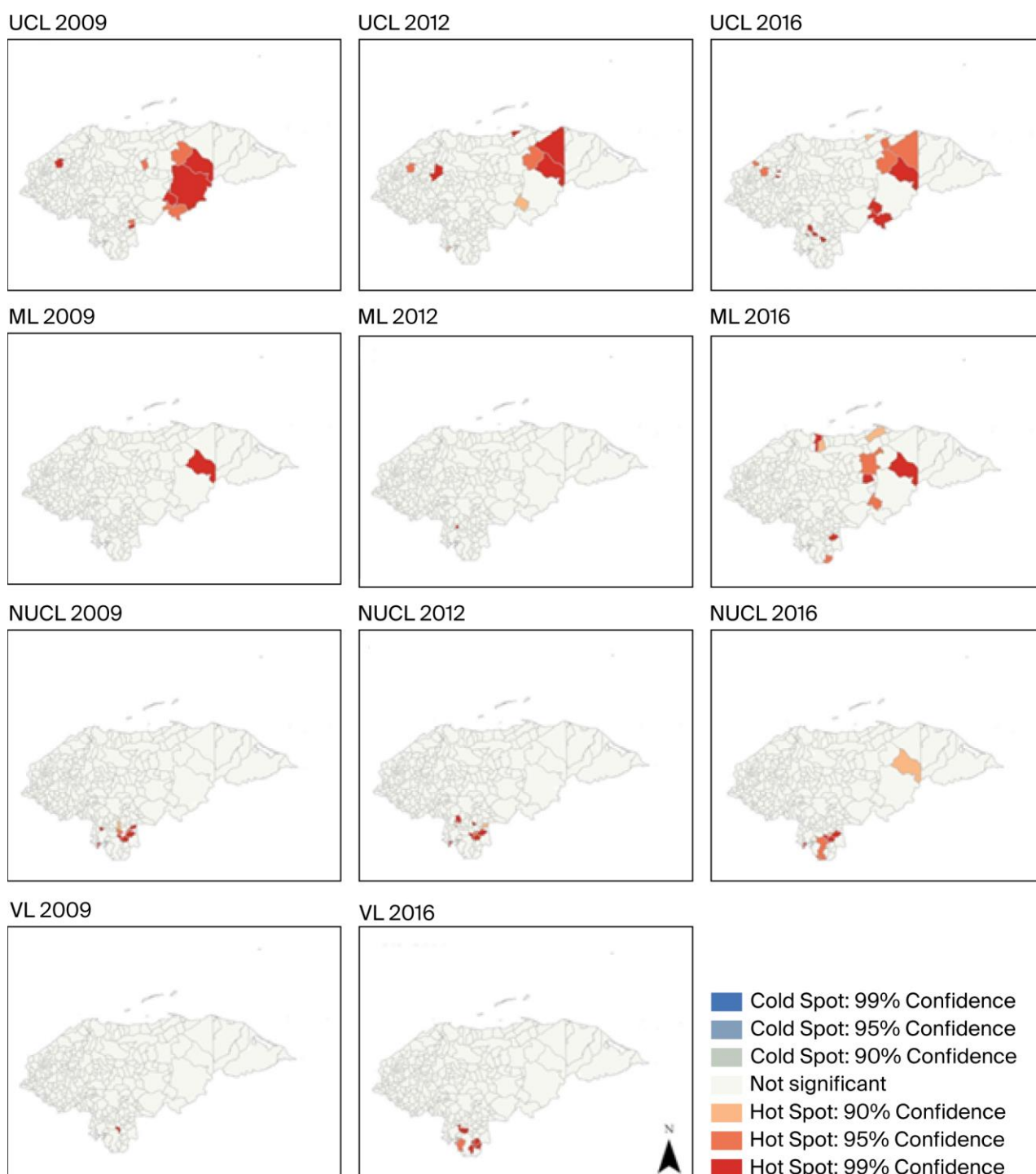


**Figure 5.** Local hot spots of four clinical forms of leishmaniasis identified using the Getis-Ord  $G_i^*$  statistic. Note: No available data for VL incidence in 2012.

#### 4. Discussion

This study provides the first description of the spatial distribution of leishmaniasis across the departments and municipalities of Honduras. An analysis of the morbidity of the four clinical forms present in the country was conducted using data from two official sources [15]. In Honduras, it is mandatory to report leishmaniasis weekly, monthly, and individually during case investigations [15]. Even so, the actual number of patients remains unknown, and the *Leishmania* species is not reported in most departments. Consequently, the absolute magnitude, potential distribution and the risks associated with the disease

are still unclear. Between 2009 and 2016, 15,345 cases of leishmaniasis were reported, primarily concentrated in Olancho, Choluteca, Colón, and Valle. The rural population was the most vulnerable, as noted in other studies [2,4,17]. A significant statistical difference was discovered ( $\chi^2 = 246.86$ ,  $p < 0.0001$ ) between males and females from 2013 to 2016, with the 20–49 years age group having the highest number of cases (38.33%). This may be attributed to the agricultural activities of men, which increase the likelihood of occurrence of the disease in this demographic, similar to findings from other studies conducted in Latin America [18–20].

During the period (2009–2016) under analysis, UCL was the predominant clinical form of the disease (60.37%), similar to the incidence in Costa Rica, Panamá, and Nicaragua [21], which are also located in Central America. The NUCL, or atypical variant of cutaneous leishmaniasis caused by *Leishmania (L.) infantum*, has been observed in Honduras [6,8,22], Nicaragua [23,24], Costa Rica [24,25], and Paraguay [26]. It was the second most prevalent clinical form of the disease in Honduras.

Our data also showed that morbidity from the disease during the study period increased by 60% compared to that during the period from 2001 to 2008 [21]. This increase was primarily due to improved health service coverage by the Honduran Health Secretary, as well as ongoing active case surveillance in endemic areas. The data presented here offer a new perspective on the distribution of various clinical forms of leishmaniasis as well as the species associated with their transmission in Honduras.

Currently, there is no reference regarding the distribution of *Leishmania* species in Honduras. Specific studies have primarily focused on the NUCL form, addressing histopathological and immunological findings [8,27–30], vector behavior [5,7,31,32], and the parasite species involved in transmission [9,22,28]. Geographically, the spatial analysis revealed segregation of the clinical forms of the disease, with higher probabilities of VL occurring in semiarid areas in the southern parts of the country, similar to reports from Nicaragua [23] and Costa Rica [25]. ML and UCL are prevalent in humid areas in the northeast, with climate zones similar to those found in Costa Rica [33,34] and Panamá [33–35]. Based on this evidence, it is essential to conduct research that correlates the incidence of clinical forms of leishmaniasis with biophysical variables.

The thematic maps in Figure 4 illustrate the spatial distribution of the incidence rate of the four clinical forms of leishmaniasis in the years 2009, 2012, and 2016, calculated per 10,000 inhabitants. Studies utilizing spatial analysis tools are relatively new, and this is the first time they have been applied in Honduras; however, reports of these tools being used in South America exist [36–38]. UCL has been present throughout Honduras in all three years, with the highest values recorded in 2016 in Patuca and Dulce Nombre de Culmí, both municipalities in the Olancho department, as well as in Trojes in the El Paraíso department. In 2012, Dulce Nombre de Culmí registered the highest rate, along with Santa Fé and Santa Cruz de Yojoa, which belong to the Colón and Cortés departments, respectively. In 2009, San Antonio in the El Paraíso department had the highest incidence, followed by Dulce Nombre de Culmí and Patuca. The analysis reveals the distribution of CL incidence in regions with similar temperature and rainfall fluctuation. Studies have been conducted across endemic countries in the New World [39–41]. The occurrence of ML was mostly localized; the municipality of Dulce Nombre de Culmí represents the primary transmission focus for this clinical form of leishmaniasis. Optimal associations have been reported between areas with the presence of CL/ML [42]. These associations may be related to optimal environments for the sandfly populations reported in the region, with less variation in humidity levels [43,44].

In 2009, the departments of Choluteca and El Paraíso exhibited remarkably high incidence rates of NUCL. The highest values were recorded in Choluteca's municipalities of

Apacilagua, Orocuina, and Morolica, along with San Francisco de Coray in Valle. The peak in 2012 shifted to Morolica, Amapala, and Reitoca from the Francisco Morazán department. By 2016, the incidence rate had significantly decreased compared to previous years, while Morolica and Orocuina also showed a decline; Amapala maintained a rate similar to its 2012 values.

Regarding the incidence of VL disease, no cases were reported for 2012. Overall, the disease did not show representative values for other years. The few records available correspond exclusively to the Choluteca department. In 2009, there were only cases in Orocuina, while in 2016, one case was reported in each of the following municipalities: Concepción de María, Santa Ana de Yusguare, Pespire, El Corpus, Namasigue, Marcovia, and Choluteca. This confirms that the incidence was highly localized to this single department and exhibited a very low rate. Similar reports have been described primarily in South American countries [37,45].

The Getis-Ord  $G_i^*$  maps from 2009 to 2016 for UCL and ML indicate hot spots mainly in the northeast of Honduras. This same index groups several focal municipalities in the south, specifically for NUCL and VL during the same period (Figure 5). No cold spots were detected, given a high percentage without statistical significance for all *Leishmania* cases in the years with recorded observations. In 2016, the sample increased in hot spots for all four clinical forms of leishmaniasis in the southern region of Honduras, along with a slight increase in municipalities with VL cases compared to those in previous years. Meanwhile, in 2012, there was a dramatic decrease in hot spot areas in the south for ML and VL. In all cases, the results are highly influenced by the weighted average of neighboring municipalities. This allows for testing the occurrence of temporal probability in the Cartesian plane and the high-high associations (hot spots) in Figure 5.

The results of the technical spatial statistics based on GIS illustrate the spatial behavior of the different forms of leishmaniasis from 2009 to 2016 in Honduras. This constitutes a baseline for future investigations regarding the biophysical factors of the terrain that can directly affect this distribution pattern, such as soil types, topography, vegetation, and regional climate, for a comprehensive analysis of the disease.

Although the methodology is based on the general principles of spatial statistics, the spatial diffusion patterns and the detection of hot spots provide valuable insights for health planning and predicting future disease behavior based on the distribution and hot spots observed over several years in Honduran municipalities. On the other hand, although our analysis describes the incidence of the disease in Honduras, we found that one of the limitations of our study lies in the uncertainty of the true magnitude of leishmaniasis, which is due to the underreporting of cases, the absence of reports on *Leishmania* species in the country, and the lack of biophysical variables for a complete understanding of the proposed spatial analysis.

## 5. Conclusions

In conclusion, findings from this study demonstrate geographical segregation among the different clinical forms of leishmaniasis, where precipitation and temperature serve as predictive factors for the distribution of the disease. Further investigations, which include *Leishmania* species typing, vector identification, and their association with different clinical forms, will enhance our understanding of the geographical distribution of leishmaniasis in Honduras.

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and E.A.H.; writing—review and editing, W.S.-O., S.A.-V., G.M.-L., C.A.R.-M., C.Z., O.N.Z., E.A.H., C.M.S.-P., G.V.A.-F. and M.D.L.; visualization, W.S.-O.; supervision, S.A.-V. and M.D.L.; project administration, W.S.-O.; funding acquisition, W.S.-O. and S.A.-V. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Consent to participate was not necessary due to (a) the absence of personal information; (b) the study's contribution to public health; and (c) the absence of any harm to the participants. The data were obtained from the Honduran Ministry of Health and do not include personal information about patients.

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## Abbreviations

|      |                                       |
|------|---------------------------------------|
| UCL  | Ulcerative Cutaneous Leishmaniasis    |
| ML   | Mucosal Leishmaniasis                 |
| NUCL | Non-Ulcerated Cutaneous Leishmaniasis |
| VL   | Visceral Leishmaniasis                |

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