

ORIGINAL ARTICLE

Epidemiological analysis of the New World screwworm (*Cochliomyia hominivorax*) in Ecuador

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Abstract

The New World screwworm (*Cochliomyia hominivorax*) is an obligate parasite that affects warm-blooded animals. It causes myiasis in livestock and humans, which is a problem for animal production and public health. The health and economic burden of myiasis on livestock production is largely unknown in Ecuador. We investigated the presence of the screwworm and analysed the epidemiology and spatial and temporal trends of myiasis in cattle farms of San Miguel de Los Bancos county. In total, epidemiological questionnaires were conducted in 110 farms, which were subsequently monitored for 12 months. The findings show that the initial and final prevalences in farms were 70% and 61.81%, respectively, and the average monthly prevalence was 15.08%. The initial and final prevalences in animals were 3.87% and 4.60% for bovines and 2.91% and 3.36% for all animals examined. The average percentage of new cases reported per month was 17.68% with a minimum of 10 and a maximum of 28 cases in October and May 2015, respectively. The cumulative incidence estimated that the risk for non-infested farms to become infested could reach 100% in approximately 6 months. The incidence rate is 168 per 1,000 farms at risk-monthly. The annual incidence was 459 per 10,000 for bovines at risk-annually. An analysis of hotspots based on the Getis-Ord Gi* index revealed no temporally stable hot spot, but one temporally stable cold spot, suggesting that most of the study area is generally favourable to infestation, except one cluster of farms.

KEYWORDS

Cochliomyia hominivorax, Ecuador, incidence, incidence rate, New World Screwworm, prevalence

1 | INTRODUCTION

The New World screwworm (NWS) fly (*Cochliomyia hominivorax* (Coquerel); Diptera: Calliphoridae) is an obligate parasite of warm-blooded animals. Infestation of tissue by fly larvae is known as myiasis (OIE, 2018). The fly is native to the Americas and, to date, has been eradicated from the United States to Panama. With the exception of Chile, the NWS remains endemic in South America and in the Caribbean countries of Cuba, Dominican Republic, Haiti, Trinidad and Tobago and Jamaica (Loker & Hofkin, 2015; Robinson,

Vreysen, Hendrichs, & Feldmann, 2009; Rodríguez, Rojas, Alvarez, & Parra, 2011).

The New World screwworm myiasis is included in the list of common diseases in domestic animal species of the World Organization for Animal Health (OIE). It is a notifiable disease in Ecuador and the Americas (OIE, 2018) and its eradication is considered a priority transboundary disease in the Americas, where it persists in many countries as a cause of concern (Domenech, Lubroth, Eddi, Martin, & Roger, 2006; FAO, 1993). This ectoparasite has been observed in tropical and subtropical areas with temperatures above 21°C, relative

humidity around 70% and the presence of hosts (Carrillo-Toro, 2015). The NWS fly has been reported at altitudes up to 2,100 masl (Spickler, 2016).

The importance of NWS includes not only the risk of myiasis to livestock but also to other domestic animals, humans, and wildlife (Rodríguez et al., 2011). NWS myiasis is associated with serious consequences for public and animal health (Grisi et al., 2014; Nascimento et al., 2005; Rodríguez et al., 2011). In livestock, myiasis lesions, symptoms, and secondary infections reduce productivity and cause stress. Prevention, control, treatment, and possible mortality constitute an important economic burden for livestock owners. These costs have been estimated to 3591.17 million USD per year in South America (Rodríguez et al., 2011); just Brazil reported losses of 336.6 million USD in 2011 (Grisi et al., 2014). Control and eradication are therefore considered necessary in South America, including in Ecuador.

In Ecuador, the ecology of the fly and the epidemiology NWS myiasis in domestic and wild animals are poorly understood (Morejón, 2015). So far, NWS myiasis has been reported in bovines, dogs, horses, and humans. While the earliest report of NWS in cattle in Ecuador was presumably made by Dr. Moya-Borja in the 1980s, Miño, Vinuesa, and Falconí (2005) reported NWS larvae in 522 farms out of 1,255 surveyed, and a total of 4,132/78,704 that is 5.25% animals were parasitized. Arteaga, Rodríguez, and Olivares (2012) found 73.4% of cattle, 13.7% of pigs, 8.8% of sheep, 5.3% of horses, 1.8% of goats, and 0.4% of canines affected by NWS myiasis in a tropical, coastal region of Ecuador. *Cochliomyia macellaria* was reported in three animals as secondary myiasis to *C. hominivorax*. In 2015, Morejón (2015) reported the presence of traumatic myiasis in 66.25% of farms in the Bolívar province with 14.62% of cattle affected. Other studies (Carrillo-Toro, 2015; Jervis, 2014; Morejón, 2015) reported *C. hominivorax* and *C. macellaria* as the only species causing multiple myiasis in Ecuador. The New World screwworm is also zoonotic and may cause myiasis in humans (Forero-Becerra, Cortés-Vecino, & Villamil-Jiménez, 2009). Chico, Cordova, Calvopiña, and Guderian (1994), Aleman and Quezada (2014), Dominguez Enriquez, Cueva Rosillo, Cusco Cuzco, Rodríguez-Hidalgo, and Calvopiña (2016), Reinoso-Quezada and Alemán-Iñiguez (2016), OIE (2018) reported cases of NWS myiasis in humans in Ecuador.

Despite several reports of NWS and myiasis in Ecuador, the epidemiological situation is poorly understood, in particular the possible confusion with other causative agents, such as *Dermatobia hominis*. This study aimed to assess the epidemiology and the temporal and spatial distributions of the NWS fly in an endemic area in Ecuador.

2 | MATERIALS AND METHODS

2.1 | Study area

The study took place in the county of San Miguel de Los Bancos (0°01'20.3"N; 78°53'41.9"W), in the western Andes foothills, 200 km from the capital Quito. The landscape is a mosaic composed of patches of pastures and of remnant cloud, tropical and subtropical

forests, mostly concentrated along the river network. A longitudinal study was conducted between December 2014 and November 2015. In total, 110 farms in 18 different communities were surveyed (Figure 1). Farms were at an average altitude of 1,250 masl (min = 550. Max = 2,000 masl), with annual mean temperatures ranging from 15 to 32°C and a mean monthly precipitation of 224 mm. The average relative humidity was around 86.42% (INAMHI) <http://186.42.174.241/InamhiPronostico/geovisor.php>.

2.2 | Data

The epidemiological questionnaire covered information on the farm and the farmer, farm coordinates, and risk factors associated with the presence of adult NWS fly, myiasis, and the use of control measures for example, bioclimatic areas, altitude, precipitation, temperature, production type, farm size, number of bovines, forest distance, and river distance. In total, 110 farmers were interviewed both in December 2014 and in November 2015. In order to measure annual and monthly apparent prevalence (AP) and incidence (I), we visited each farm monthly to record NWS myiasis cases. A farm was considered positive if at least one case of NWS myiasis was reported. Unfortunately, we could only confirm a small proportion of cases in the lab, those that were identified following a notification by the farmer. All other cases were clinically assessed after farmer's observation that is multiple larvae, eggs lays in natural or man-made wounds, *C. hominivorax* larvae feeding on living flesh, producing annoyance, pain, discomfort, and bad smell. As there are no other species of wound-infesting insects in Ecuador (Carrillo-Toro, 2015), we are confident in the distinctive clinical diagnosis. Case information included farm coordinates, species affected, breed, age, myiasis signs, myiasis localization on the body, myiasis cause and treatment measures, if any.

2.3 | Epidemiological parameters

We computed the apparent and true prevalence (TP), the incidence (I), the incidence rate (IR) and the cumulative incidence (CI).

Considering that prevalence is the amount of disease observed at a specified time, (Jaramillo & Martínez, 2010; Thrusfield, 2007), we computed the AP as the proportion of positive animals, regardless of their true status for the disease in question. Of all animals assessed as positive, some are likely false positives (Jaramillo & Martínez, 2010; Thrusfield, 2007). We computed the AP for farms and animals as:

$$AP = \frac{\text{number of individuals having a disease at a particular point in time}}{\text{number of individuals in the population at risk at that point in time}} \quad (1)$$

In the absence of a gold standard, inaccurate tests characteristics (Se and Sp) and sampling variability affect sometimes the measured prevalence of a disease. Hence, a Bayesian approach can be useful as it allows flexibly combining the available "prior" knowledge (expert opinion) on diagnostic test characteristics. This has a great impact on the final estimation of the true prevalence. For this

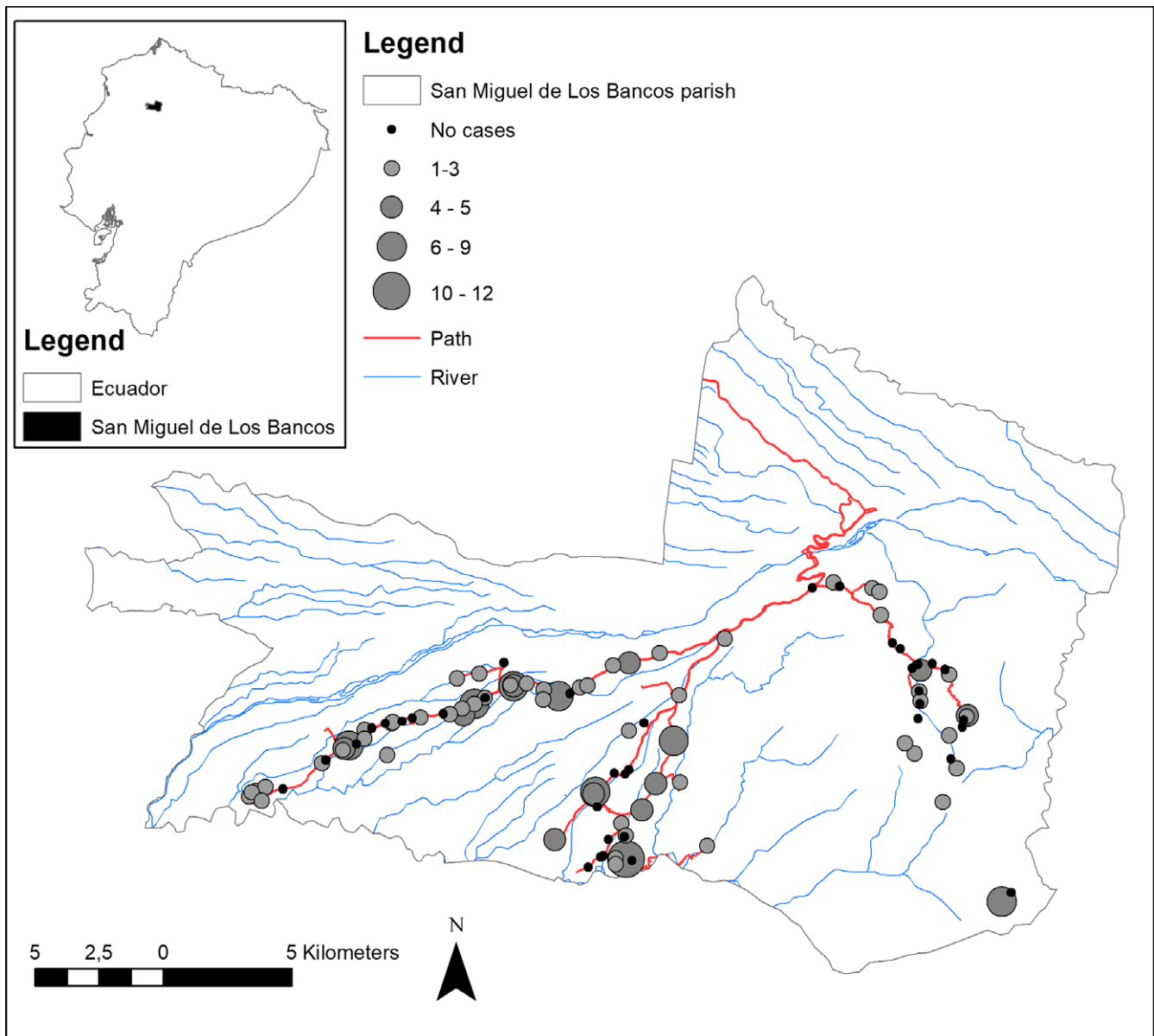


FIGURE 1 Study area and the New World screwworm incidence over December 2014–November 2015 [Colour figure can be viewed at wileyonlinelibrary.com]

reason, we computed TP using a multinomial Bayesian model (Berkvens, Speybroeck, Praet, Adel, & Lesaffre, 2006; Praet et al., 2013; Speybroeck, Devleeschauwer, Joseph, & Berkvens, 2013). Prior information on sensitivity and specificity were extracted from Simonovis, Delgado, Piras, Pulido, and Llatas (2007), that is between 55% and 66% for sensitivity and between 98% and 100% for specificity. We computed the TP for farms and animals as:

$$TP = \frac{\text{Apparent prevalence} + \text{Specificity of the test} - 1}{\text{Sensitivity of the test} + \text{Specificity of the test} - 1} \quad (2)$$

Incidence was defined as the number of new cases occurring in a known population over a specified period of time (Jaramillo & Martínez, 2010; Thrusfield, 2007). Cumulative incidence (also termed risk) was defined as the proportion of non-diseased individuals at the beginning of a period of study that become diseased during the

period (Jaramillo & Martínez, 2010; Thrusfield, 2007), and computed for farms and animals as:

$$CI = \frac{\text{number of individuals that become diseased during a particular period}}{\text{number of healthy individuals in the population at the beginning of that period}} \quad (3)$$

Finally, the IR measured the rapidity with which new cases of disease develop over time (Jaramillo & Martínez, 2010; Thrusfield, 2007), and was computed for farms and animals as:

$$IR = \frac{\text{number of new cases of disease that occur in a population during a particular period of time}}{(\text{number at risk at the start of the time period} + \text{number at risk at the end of the time period}) / 2} \quad (4)$$

TABLE 1 Annual apparent prevalence and true prevalence of myiasis in the county of San Miguel de Los Bancos

	Year	Positives	Population	Apparent prevalence (CI _{0.95})	True prevalence (CI _{0.95})
Farm ^a	2014	77	110	0.70 (0.60–0.77)	0.97 (0.88–0.99)
	2015	68	110	0.62 (0.52–0.70)	0.93 (0.81–0.99)
Bovine ^b	2014	166	4288	0.04 (0.03–0.05)	0.05 (0.03–0.07)
	2015	197	4278	0.05 (0.04–0.05)	0.06 (0.04–0.08)

Notes. CI_{0.95}: confidence interval 95%.

^aAll farms were included in the analysis; ^bAll bovines were included in the analysis; 2014.

2.4 | Temporal analysis

Apparent prevalence, CI and IR were analyzed over time (Jaramillo & Martínez, 2010; Thrusfield, 2007) for the entire period as well as monthly. TP computation and statistical analyses were performed in R version 3.2.2 using “prevalence” and “epitools” packages (Aragon, 2017; Devleeschauwer et al., 2015; Praet et al., 2013; Speybroeck et al., 2013).

2.5 | Spatial analysis

For spatial analysis, all geographical data were transformed into the coordinate reference system EPSG:32717, WGS84/UTM zone 17S. The farms were overlaid on maps of altitude, rainfall, and temperature from official institutions (Instituto Geográfico Militar (IGM) www.geoportaligm.gob.ec, Ministerio de Agricultura y Ganadería (MAG) www.geoportal.agricultura.gob.ec and Instituto Nacional de Meteorología e Hidrología (INAMHI) <http://186.42.174.241/InamhiPronostico/geovisor.php>). Each variable was sliced into three classes. Altitude (<1,000 masl, between 1,000 and 1,600 masl and >1,600 masl), average annual rainfall (2,500–3,000 mm³, 3,000–4,000 mm³ and 4,000–5,000 mm³), and average annual temperature (between 12°C to 14°C, 20°C to 22°C and 24°C to 26°C). The associations between disease and temperature, humidity and precipitation were analysed using a Chi² (Microsoft Excel 2018 for Mac). The spatial structure of positive farms was assessed using the Getis-Ord Gi* index and a binary indicator of infestation at the farm level, computed over the entire study period and over the four trimesters making up the study period. The spatial analyses were completed in ArcGIS (Esri, ArcGIS Release 10.4, esri.com).

3 | RESULTS

At the onset of the survey, in December 2014, 77 of 110 farms surveyed declared having had at least one case of NWS myiasis in the past year. At the end of our 12-month survey, we had recorded 68 farms with at least one case. Amongst the 77 farms that reported at least one case of NWS myiasis during the year 2014, 174 infested animals were reported, including 166 bovines (*Bos taurus*), three dogs (*Canis familiaris*), two pigs (*Sus scrofa*), two goats (*Capra hircus*) and

one hen (*Gallus gallus*). At the end of 2015, amongst the 68 positive farms, 207 infested animals were reported, including 197 bovines, seven dogs and three pigs. Farmers reported lesions at the neck (44%), legs (32%), ears (28%), navel (26%) and udder (18%), principally. How the animals got infested was unknown in 50% of the cases, followed by wounds, tick bites, and warts in 45%, 25%, and 20% of cases, respectively.

3.1 | Temporal analysis

3.1.1 | Prevalence

In farms

The annual AP per farm in 2014 (as per farmers' declarations) and 2015 (as per our observations) was 70% (77/110; CI_{0.95} = 61.4%–78.5%); and 61.81% (68/110; CI_{0.95} = 52.4%–70.3%), respectively. True prevalence reached 97% and 93% respectively for each period. (Table 1; Figure 1).

The monthly farm AP average was 15.08% with a minimum of 8.57% (8/110 farms) in September 2015 and a maximum of 22.86% (24/110 farms) in May 2015. The AP decreased from December 2014 to November 2015. The monthly AP (Figure 2 and Table 2) indicated that NWS myiasis had a linear decreasing tendency over time, but this was not significant (*p*-value = 0.27).

In bovines

The annual AP, in 2014, was 3.87% (166/4,288; CI_{0.95} = 3.33%–4.49%) and in 2015, it reached 4.60% (197/4,278; CI_{0.95} = 4.1%–5.3%). True prevalence was not significantly different compared to the AP on bovines (Table 1). On average 16 new bovines got infested each month. The monthly AP was 37.31 cases per 10,000 bovines (16/4,288; CI_{0.95} = 22.46–61.10). The site and origin of myiasis and other skin lesions are described in Figure 3a,b.

3.1.2 | Incidence

In bovines

Two peaks in incidence were recorded, in January 2015 (22 cases) and in May 2015 (28 cases). In other months, between 10 and 19 cases were recorded (Table 2). The incidence slightly, linearly decreased over time, but this was not significant (*p*-value = 0.27; Figure 2).

3.1.3 | Cumulative incidence

In farms

The CI estimated that the risk for non-infested farms to become infested could reach 100% in approximately 6 months. In one year, an increase of new cases was progressively observed. The risk of one farm to become infested by NWS myiasis is 2.2 times per year.

The results of the 12-month monitoring are presented in Table 2 and Figure 4 and compared by number of farms and by total affected animals.

3.1.4 | Incidence rate

The IR per farm was 168 positives per 1,000 farms at risk-monthly. That is farms moved from NWS-free to NWS-infested at a rate 16.8% each month. About six per 1,000 farms would have at least one new case of myiasis per day. The IR in bovines was of 459 per 10,000 bovines at risk-annually (Table 3). The risk of a bovine becoming infested was around two animals per 10,000 bovines each day.

3.2 | Spatial analysis

The Gi* analysis for 12 months (Figure 5) or by trimesters (Figure 6) showed the existence and persistence of a cold spot (cluster of non-infested farms) in the east of the study area. A hot spot was detected at the center of the study area (90% confidence interval) when looking at the entire survey (Figure 5), but it only appeared in Trimester 1 (December-February). Other, non recurring, hotspots were detected in separate trimesters.

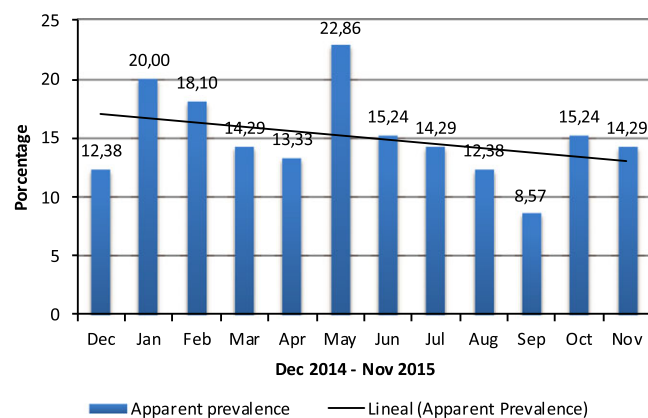


FIGURE 2 Monthly apparent prevalence of myiasis on 110 farms [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Number of cases and percentage of incidence and cumulative incidence in the study area

Month	Dec 14	Jan 15	Feb 15	Mar 15	Apr 15	May 15	Jun 15	Jul 15	Aug 15	Sep 15	Oct 15	Nov 15
Incidence ^a	16	22	19	16	15	28	17	16	15	10	16	17
Cum inc. ^c	-	38	57	73	88	116	133	149	164	174	190	207
Incidence ^b	13	21	19	15	14	24	16	15	13	9	16	15
Cum inc ^c	-	30.91	48.18	61.82	74.55	96.36	110.91	124.55	136.36	144.55	159.09	172.73

^aIncidence and cumulative incidence per cases. ^bIncidence and cumulative incidence per farm (110). ^cCumulative incidence.

Figure 7 shows the results of myiasis compared to monthly mean temperatures, humidity, and precipitation (as reported by INAMHI, <http://186.42.174.241/InamhiPronostico/geovisor.php>) in the study area. Temperature and humidity were constant throughout the year. However, precipitation varied throughout the year, being highest from December to April and lowest between May and November (Figure 7). None of these variables or altitude were significantly associated with the presence of myiasis in the area.

4 | DISCUSSION

4.1 | Temporal analysis

Our findings and previous studies confirmed the importance of NWS myiasis in tropical and subtropical areas of Ecuador. In this

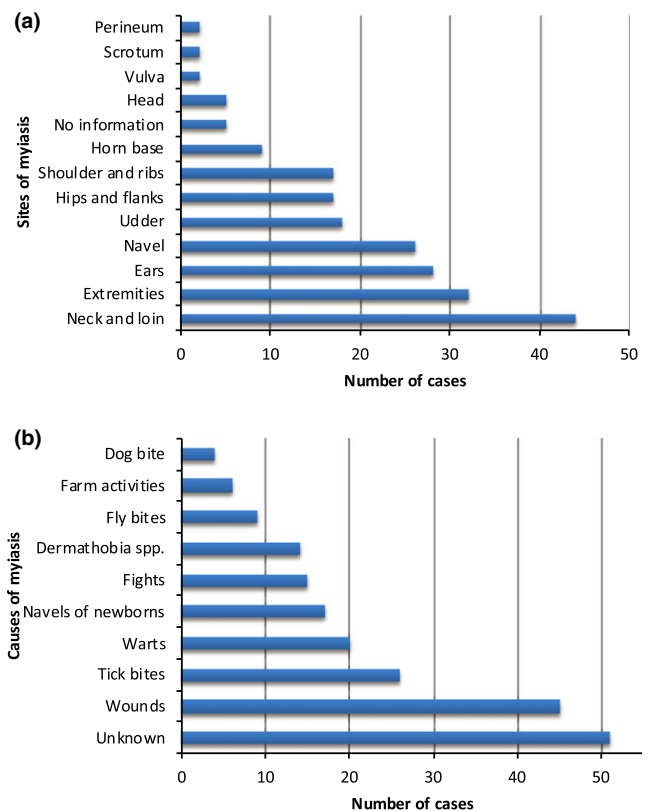


FIGURE 3 (a) Sites and causes of myiasis in bovines during the period of study. (b) Sites and causes of myiasis in bovines during the period of study [Colour figure can be viewed at wileyonlinelibrary.com]

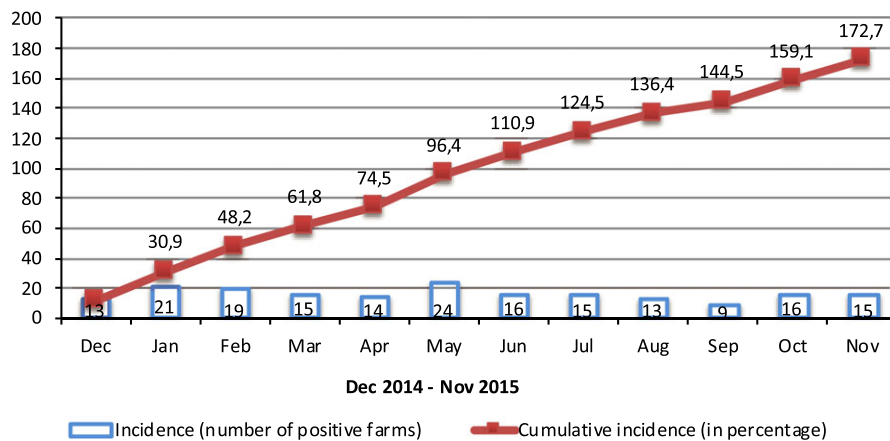


FIGURE 4 Incidence and cumulative incidence in percentages per month and per farm [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Incidence rate: Risk analysis per unit of time with relationship to the population

	Unit of time			
	Annually	Monthly	Weekly	Daily
Farm	2016 × 1,000	168 × 1,000	42 × 1,000	6 × 1,000
Bovine	459 × 10,000	38.2 × 10,000	9.56 × 10,000	1.36 × 10,000

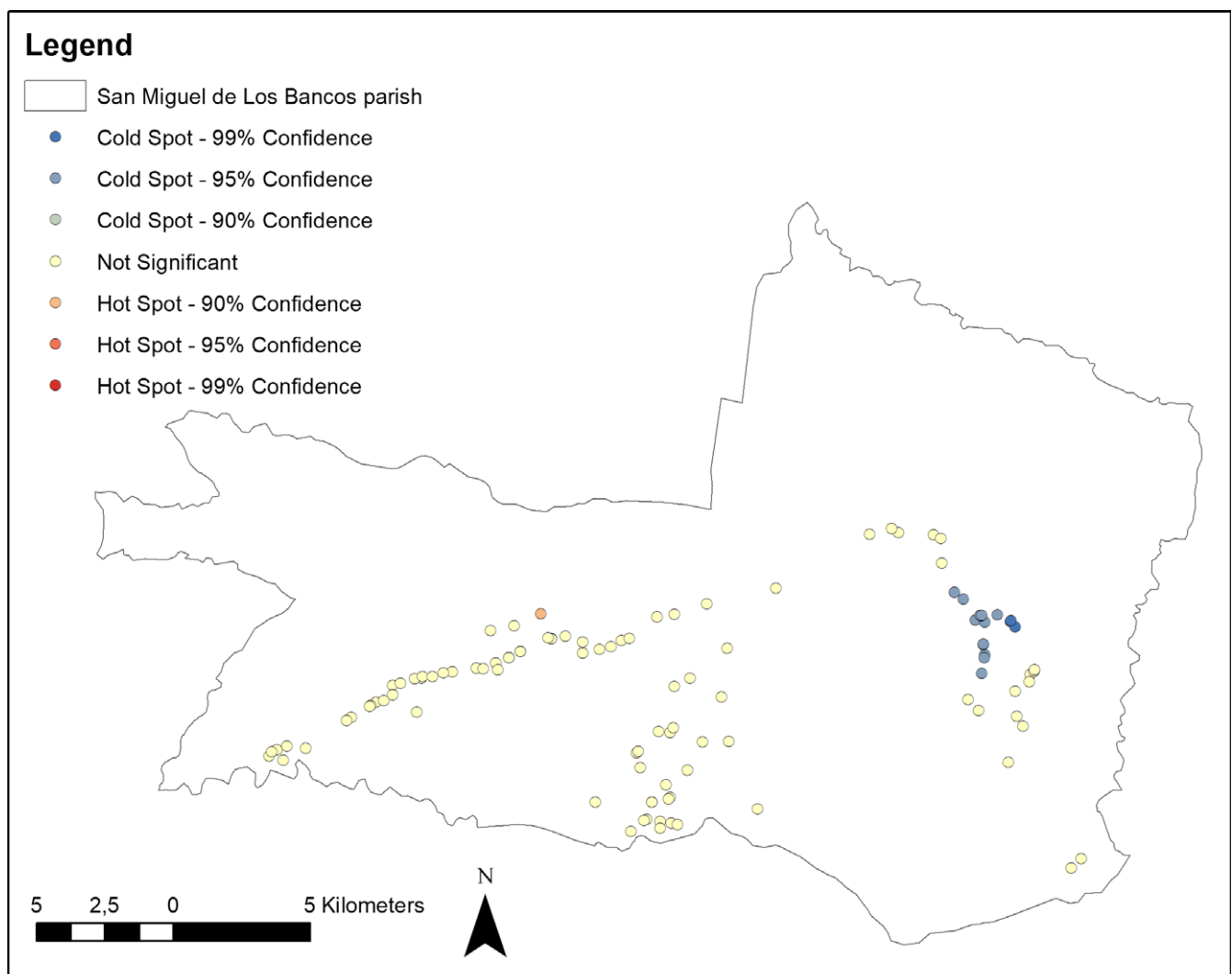


FIGURE 5 Spatial clustering analysis (Gi*) of positive farms over the entire study period (December 2014–November 2015) [Colour figure can be viewed at wileyonlinelibrary.com]

Legend

- San Miguel de Los Bancos parish
- Cold Spot - 99% Confidence
- Cold Spot - 95% Confidence
- Cold Spot - 90% Confidence
- Not Significant
- Hot Spot - 90% Confidence
- Hot Spot - 95% Confidence
- Hot Spot - 99% Confidence

10 5 0 10 Kilometers

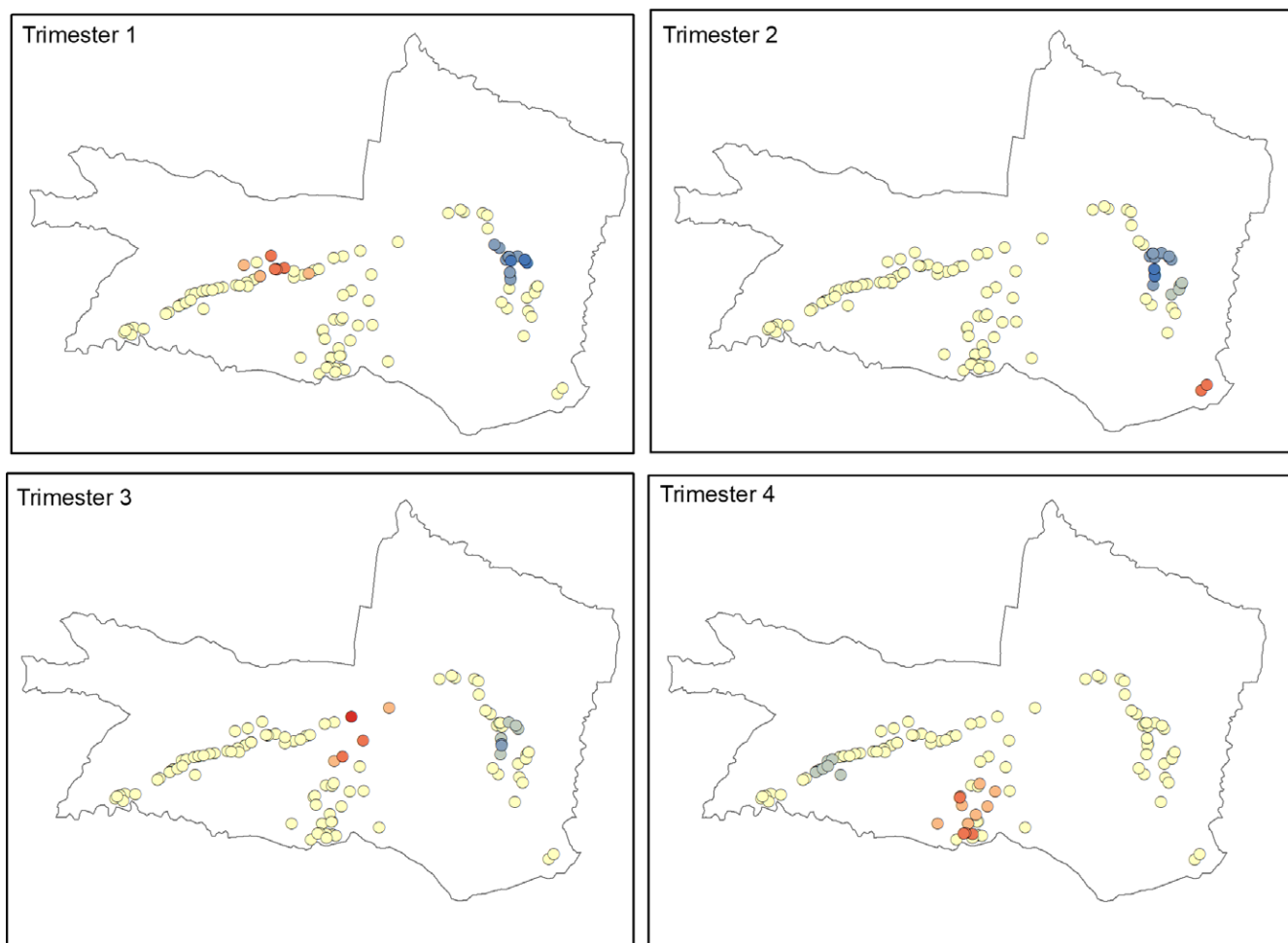


FIGURE 6 Spatial clustering analysis (Gi*) of positive farms over trimesters between December 2014–November 2015 / Trimester 1: December 2014–February 2015; Trimester 2: March 2015–May 2015; Trimester 3: June 2015–August 2015; Trimester 4: September 2015–November 2015 [Colour figure can be viewed at wileyonlinelibrary.com]

study, the AP at farm level was 70% and 62% for 2014 and 2015, respectively (Table 1). The estimated TP was 97% and 93% (2014 and 2015 respectively; Table 1). Similar results were found by Mor ejón (2015) in the Bolívar province of Ecuador with an AP of 66.25%. Similarly, in a study conducted by Baranenko et al. (2009) in pig production systems in Venezuela, the AP of NWS myiasis

reached the highest levels between December (60%) and January (50%). In addition, Miño et al. (2005) reported that the AP was 41.5% (522/1,255) at the national level, as measured by a questionnaire.

AP in bovines was on average 4.23%, which was similar to the TP calculated by Bayesian analysis. The annual AP measured by

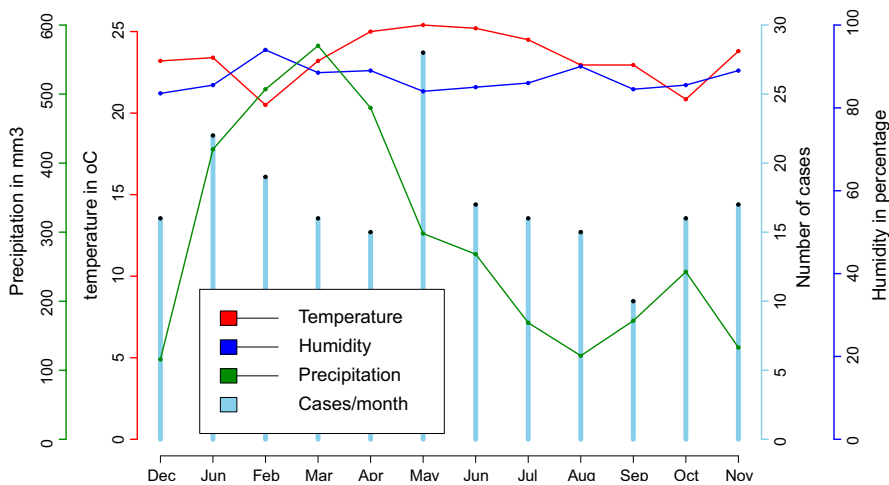


FIGURE 7 Monthly averages of temperature, humidity, and precipitation versus number of cases of myiasis [Colour figure can be viewed at wileyonlinelibrary.com]

Morejón (2015) in Bolívar province was 14.62% (1,360/9,296). This is four times higher than the AP reported in this study. It may relate to the fact that the study of Morejón was cross-sectional and ours was longitudinal. Also, the average number of bovines per farm was 20 in Bolívar and 54 in our study. In Ecuador, farms with fewer than 20 animals are considered traditional farms, which, in general, have poorer management practices that could increase the risk of infestation (Morejón, 2015).

The monthly AP and the I (Figures 2 and 4 and Table 2) suggested a slight linear decrease over time, but this was not significant. A study carried out in a barrier zone from Panama with no natural population of screwworm showed a similar trend (Maxwell et al., 2017). Although not significant, this may indicate that infestation rates could be cyclical over the years, but confirming this would require a longer follow-up. Two epidemic peaks were observed that is one in January and another in May of 2015, probably related to the seasonal use of acaricides for tick control (Bustillos, Carrillo, Jacho, Enríquez, & Rodríguez, 2015), or suggesting that occasional outbreaks or lapses in control are more common in this season. The inadequate or indiscriminate use of acaricides may have negatively affected the development of NWS fly in this study area (data not available) and further studies are needed to estimate this condition. Benítez Usher et al. (1997) and Lima, Malacco, Bordin, and Oliveira (2004) observed such effect of acaricide on NWS.

The CI increase regularly very with no noticeable step increase (red line, Figure 4). Myiasis lasted around 7 days from appearance to elimination, as per farmers' reports. All myiasis cases, according to farmers, were treated with insecticides including ivermectin, chlorpyrifos, dichlorvos or cypermethrin associated with creolin, gentian violet, iodine or even spent oil and gasoline, achieving 100% of healing success. Therefore, any affected animal, once treated and cured, became a new susceptible bovine at risk of NWS myiasis. While only two cases of NWS re-infestation were reported during the study, on different body sites from the original infestation, the CI rate per farm (Figure 4) suggests that any non-infested farm could become infested over approximately six months.

4.1.1 | Incidence rate

This study indicated that the IR per farm and per animal was 16.8% monthly and 459 per 10,000 cattle at risk-annually of NWS myiasis, respectively. In general terms, it is estimated that about six farms per 1,000 would be infested per day with at least one case of myiasis. Within a population of 10,000 animals, the risk of observing myiasis is about one animal each day. This indicates that myiasis is an important parasitic disease in Ecuador and further studies are needed to estimate its economic burden.

4.2 | Spatial analysis

According to Forero-Becerra et al. (2009), the optimal conditions for *C. hominivorax* to deposit the eggs are temperatures above 16°C, humidity between 70% and 90% and precipitation between 1,800 and 4,000 mm. The fly is commonly observed below 2,100 masl. Conditions for the NWS are therefore generally adequate throughout San Miguel de Los Bancos county, and no significant association of myiasis with altitude, precipitation, humidity and temperature could be detected. In addition, Luna, Castillo, Mancilla, Cigarruista, and Giron (2000), indicate that sources of water favour the presence of *C. hominivorax*, so that the extensive hydrography system of San Miguel de Los Bancos also provides a stable environment for the fly. The cold spot detected in the east of the area corresponds to the highest, coldest and driest area, suggesting that only that section of the study area has unsuitable, or less suitable, conditions for the fly. However, the environmental data used here were coarse, likely not including enough temporal and spatial detail to assess the environmental variability over this comparatively small study area. Maxwell et al. (2017) found no association between NWS cases and seasonality likely due to a minimal seasonality in climate in their study carried out in Panama; on the contrary, no clearly marked seasonality in the presence of NWS was noted in San Miguel de Los Bancos. Phillips, Welch, and Kramer (2004) reported that flies were most abundant during the transition from wet to dry season. This hypothesis could not be confirmed here because San Miguel de Los Bancos has stable

climatic conditions throughout the year. In our study area, the development of myiasis may be more influenced by transhumance (long distance movements along humans), animals roaming free through the landscape, rangeland damage, and traumas (personal observations). The existence of hot spots may also reflect processes of diffusion through local fly dispersion. While livestock management practices could affect the risk of infestation, farms within flight range of each other offer the fly easy spreading opportunities, possibly helped by rivers connections. This suggests that efficient control should not only target infested herds but also all neighboring ones.

In conclusion, the epidemiological parameters obtained demonstrate the importance of the NWS fly for both farms and animals and allow us, for the very first time, to assess the incidence of this transboundary disease in a tropical area in Ecuador. This information could be extrapolated to the rest of the country and the region. While climate and altitude were not found to affect the presence of NWS myiasis, the presence of a persistent cluster of low values suggests that some areas had conditions less suitable to NWS. Ecuadorian authorities can use this information to propose a suitable NWS control program. These results could underpin future epidemiological studies to improve new regional control strategies leading to the eradication of the fly in South America. Finally, further studies should be carried out to determine the geographical distribution of the NWS and to assess the fly genetic variability in different ecological niches to enhance the control programs.

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