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Spatial analysis of vampire bat (*Desmodus*) transmitted rabies virus outbreaks in livestock across Peru

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Environmental Health
2017

Abstract

Spatial analysis of vampire bat (*Desmodus*) transmitted rabies virus outbreaks in
livestock across Peru
By Amanda Vincent

Purpose: The thesis research investigated the spread of vampire bat transmitted rabies
virus in livestock across Peru using reported outbreak data from 2003 to 2016. The thesis
research aimed to identify spatio-temporal clusters of rabies outbreaks in livestock,
identify the most likely outbreak origins of the spatio-temporal clusters, and estimate the
rate at which vampire bat rabies is moving across the landscape in identified outbreak
clusters.

Methods: SatScan software was used to identify spatio-temporal clusters of outbreaks
and simple linear regression analysis was used to find the most likely outbreak origins in
clusters and to estimate the weekly rates of rabies spread in clusters.

Results: A total of 8 clusters were identified in a confirmed outbreak dataset and 13 in a
suspected outbreak dataset. The regression analysis run on suspected outbreak clusters at
the week level estimated rates of spread spanning from 0.22-5.03 kilometers per week
and the estimated rates of spread for the confirmed outbreak clusters ranged from 0.33-
6.68 kilometers per week.

Conclusions: The outbreak distributions within clusters and r^2 values indicated the
presence of wavefronts of rabies spread across the landscape. The research can be used to
aid vampire bat transmitted rabies control strategies by providing targeted spatial
identification and forecasting of existing and future outbreaks in livestock. The potential
use of this research in vampire bat rabies control will promote public health by aiding
prevention of spillover infections in humans.

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Acknowledgements

Table of Contents

Introduction

In Latin America, vampire bat transmitted rabies virus is considered a re-emerging disease with major public health and economic implications (Benavides, Valderrama, & Streicker, 2016; WHO, 2013). Human infections with vampire bat transmitted rabies have been increasing in South America over the past decade (Condori-Condori, Streicker, Cabezas-Sanchez, & Velasco-Villa, 2013). Peru is of particular concern, as recent research has found evidence of wave front rabies virus spread across parts of the landscape (Streicker, Winternitz, Satterfield, Condori-Condori, Broos, Tello, . . & Valderrama, 2016; Benavides et al., 2016). Many experts identify rabies to be "the most important viral zoonosis," because of its extreme mortality rate and spillover potential (Hanlon, Niezgoda, & Rupprecht, 2007, p. 201; Real, Russell, Waller, Smith, & Childs, 2005; Childs & Real, 2007). Wave front spread of rabies virus in Peru presents an apt example of an ecological and interdisciplinary public health problem, requiring the unification of resources and knowledge from human health, animal health, and disease ecology.

Rabies is a very old viral zoonotic disease that causes encephalitis, or brain and spinal cord inflammation, and death in humans and animals (Heymann, 2015; Fooks, Banyard, Johnson, McElhinney, & Jackson, 2014; Streicker et al., 2016). While common understanding conjures images of aggressive canines frothing at the mouth, rabies can present as either furious or dumb (Hanlon et al., 2007). Furious rabies is the aggressive stereotype, while dumb rabies presents more paralytic symptoms (Hanlon et al., 2007). In

general, humans, bats, and cattle present with paralytic clinical rabies (Kuzmin & Rupprecht, 2007).

In addition to being extremely deadly, rabies is extremely adaptive and virtually all mammals can become rabies hosts, many of which are carnivores (Childs & Real, 2007). However, in order for a rabies host to become a reservoir, the rabies virus variant must adapt to the reservoir species (Hanlon et al., 2007). While there are many rabies variants capable of infecting new hosts, such spillover infections often result in dead-end infections or hosts (Childs & Real, 2007; Hanlon, et al., 2007; Real et al., 2005). Livestock and humans in particular are dead-end rabies hosts, given that neither makes a biological contribution to the disease maintenance. In Latin America, the main rabies reservoirs are canines and bats Heymann, 2015; Streicker et al., 2016; Benavides, Valderrama, & Streicker, 2016).

Background: Rabies Epidemiology

Rabies is from the genus *Lyssavirus*, which consists of 14 virus species, and the family *Rhabdoviridae* (Wunner, 2007; Banyard, Evans, Luo, & Fooks, 2014). A rabies case is considered to be any infection from the Lyssavirus family (Childs & Real, 2007). All but two of the Lyssavirus species have been found in bat species (Banyard et al., 2014). Only classical rabies virus (RABV, genus *Lyssavirus*) is currently endemic in the Americas, and RABV is the only lyssavirus found in the New World (Heymann, 2015; Banyard et al., 2014). Only bats found in the Americas carry RABV and the species most often involved in fatal bat to human RABV transmission include: big brown bats,

Mexican or Brazilian free-tail bats, silver-haired bats, tri-colored bats, and vampire bats (Banyard et al., 2014).

Rabies carries a nearly 100% case-fatality rate, and recovery in both animals and humans is rare (Baer and Olsen, as cited in Hanlon et al., 2007; Heymann, 2015; Fooks, Banyard, Johnson, McElhinney, & Jackson, 2014; Streicker, Winternitz, Satterfield, Condori-Condori, Broos, Tello, Recuenco, Velasco-Villa, Altizer, & Valderrama, 2016). The estimated global human rabies mortality is between 26,000 to 61,000 deaths per year (WHO, 2013). While these rabies numbers are not as staggering as other diseases, it is important to note that there is widespread underreporting of rabies cases, especially bat transmitted rabies cases (Hanlon et al., 2007; Hampson, Coudeville, Lembo, Sambo, Kieffer, & Attlan, 2015). Furthermore, the complexities of rabies control and elimination come with a high economic burden, which is especially evident in Latin America (WHO, 2013; Hanlon et al., 2007, Hampson et al., 2015). According to the Pan American Health Organization (PAHO), as of 2013, in Latin America and the Caribbean, canine rabies control and elimination campaigns alone required an annual budget of US$20 million (WHO, 2013). In Latin America, an estimated 70 million cattle are considered at risk for bat transmitted rabies infection (Kuzmin & Rupprecht, 2007; Johnson, 2014). However, a study by Hampson et al. (2015) found a level of uncertainty present in global rabies estimates due to insufficient surveillance practices.

Vampire bat ecology

The common vampire bat is one of Latin Americas main rabies virus reservoirs and the first reported vampire bat transmitted human case in the Americas occurred in 1927 in Trinidad (Heymann, 2015; Streicker et al., 2016; Benavides et al., 2016;

Schneider, Romijn, Uieda, Tamayo, da Silva, Belotto, & Leanes, 2009). In Latin America, vampire bats are reservoirs for the sylvatic rabies type, while domestic dogs are reservoirs for urban rabies (Condori-Condori et al., 2013). Common vampire bats (*Desmodus rotundus*) are only found in the Americas, ranging from Mexico to Argentina, and are the most established rabies reservoir among hematophagous or blood-feeding bats (Heymann, 2016; Johnson, 2014). Blood-feeding makes them successful rabies sources or transmitters (Johnson, 2014). Vampire bats transmit rabies by biting prey and contaminating the bite wound with infected saliva (Johnson, 2014; George et al., 2011). Their preferred prey is livestock, especially cattle, but they also feed on wildlife, humans, and smaller livestock (Johnson, 2014; Streicker & Allgeier, 2016). Vampire bats always feed at night and their bite has very little pain (Johnson, 2014; Streicker & Allgeier, 2016). This makes vampire bats successful blood-feeders because livestock owners are less likely to notice vampire bats feeding on their livestock due to the timing of bites and the lessened distress caused by bites with little pain (Johnson, 2014; Streicker & Allgeier, 2016).

While vampire bats are rabies reservoirs, not all bats become infectious (Bell, 1964; Kuzmin & Rupprecht, 2007). In a study by Moreno and Baer, it was shown that the bats could develop clinical infection and die shortly after symptoms presented, whereas bats that survived inoculation were not infectious (as cited in Kuzmin & Rupprecht, 2007). When individual infected bats do not become infectious and die, it is an abortive infection (Bell, 2964; Kuzmin & Rupprecht, 2007). Bats become inoculated from close social contact with infected fellow roost bats, via contaminated saliva (Kuzmin & Rupprecht, 2007). As a RABV reservoir, vampire bats are mammals capable of viral

maintenance of the rabies variant in a given geographical area (Hanlon et al., 2007). While vampire bats are one of the main rabies reservoirs in Latin America, RABV is not always present in a given population (Streicker et al., 2016; Benavides, 2016). This is due to trends in viral transmission and maintenance, which influence its spread (Childs & Real, 2007; George *et. al*, 2011). The maintenance cycle in vampire bats involves, to the best understanding in the literature, introducing the virus into a naïve or uninfected bat or bat population, resulting in infection and viral multiplication, which ends with the new reservoir bat re-starting the cycle with other susceptible bats (Blackwood, Streicker, Altizer, & Rohani, 2013). For rabies maintenance to function, the virus must be exposed to an uninfected individual belonging to the original reservoir species (Childs & Real, 2007).

Livestock in Peru

In Peru, cattle represent the largest portion of livestock ownership, followed by sheep and goats, poultry, and lastly pigs (FAO, 2005). National livestock distribution naturally follows a similar distribution to human population density (FAO, 2005). Much of Peru's livestock are part of small farms, located in the highlands where there is adequate grassland for feeding (FAO, 2005). While cattle ownership is widespread in Peru, most cattle are not used for meat production, but for dairy (FAO, 2005). Given that cattle ownership is concentrated in small scale farms (FAO, 2005), it is likely that cattle are relied upon for household dairy subsistence, in addition to dairy production.

Rabies research commonly uses livestock rabies cases as a proxy for vampire bat presence (Streicker et al., 2016; Benavides et al., 2016), due to difficulties in tracking bat

movements (Streicker et al., 2016; Benavides et al., 2016; Johnson et al., 2014). Given that livestock dead-end RABV hosts and are the preferred prey of vampire bats, they make suitable sentinels for monitoring RABV circulation in bat populations (Streicker & Allgeier, 2016; Streicker et al., 2016).

Viral invasion and seasonal expansion

Throughout rabies research, specific trends are consistently identified as drivers of viral invasion and seasonal expansion of bat transmitted lyssaviruses, including: seasonality, geographic distribution of bat hosts, anthropogenic change, and livestock density (Streicker et al., 2016; Streicker & Allgeier, 2016; Streicker, Lemey, Velasco-Villa, & Rupprecht, 2012; Benavides et al., 2016; George, Webb, Farnsworth, O'Shea, Bowen, Smith, & Rupprecht, 2011). Vampire bats present several difficulties to efforts to monitor RABV in vampire bat populations, including: wide geographic distribution, isolated roosting sites, and flight ability (Stoner-Duncan, Streicker, & Tedeschi, 2014; Streicker et al., 2016; Benavides et al., 2016; Johnson et al., 2014). As a result, spatial modeling and mapping are often used to investigate the incidence and transmission of RABV to animals and humans, as well as to extricate the complexities of RABV viral evolution (Smith et al., 2002; Real et al., 2005; Blackwood et al., 2013; Benavides et al., 2016; Streicker et al., 2016). Viral invasion and seasonal expansion of RABV in Peru is interdependent, as RABV variants invade new vampire bat populations via seasonal expansion of male dispersal from the host populations (Streicker et al., 2016; Blackwood, Streicker, Altizer, & Rohani, 2013). However, whether or not invading RABV variants are maintained in naïve bat populations is determined by susceptibility in the population

and (Streicker et al., 2016; Blackwood, Streicker, Altizer, & Rohani, 2013). Rabies variants are species specific, and while geographic distribution is a known factor, there is currently no mechanistic explanation for the variability in geographic distribution (Banyard et al., 2014).

Streicker et al. (2016) and Benavides et al. (2016) have completed significant work on vampire bat rabies invasion and expansion trends in Peru by using livestock as proxies, into the avenues of identifying landscape barriers to rabies spread, evidence of seasonal expansion, and predicting rabies spread into new areas in Peru's landscape. The research has demonstrated consistent trends of vampire bat dispersal expansion and rabies virus invasion into areas where rabies was historically absent (Streicker et al., 2016; Benavides et al., 2016). These expansions and invasions across the landscape are increasingly considered to be waves of spread, and this wave front process has been observed in other infectious diseases, such as Ebola (Benavides et. al, 2016; Walsh, Biek, & Real, 2005). It is predicted that vampire bat rabies virus will invade Peru's coast by 2020 (Streicker et al., 2016). This trend of expansion identifies vampire bat rabies as an emerging disease requiring novel rabies control strategies (Benavides et al., 2016).

Benavides et al. (2016) also explored elevation as a landscape barrier to rabies expansion in Peru and found that overall, RABV was expanding into the Andes and elevation levels of outbreaks were increasing. Furthermore, Benavides et al (2016) found wavefront spread in the landscape and concluded that the speed of waves traveling through valleys suggest RABV spread in vampire bats populations occupying constrained areas more so than expanding into new territories via flight (Benavides et al., 2016). Using landscape factors to investigate barriers and promoters of RABV spread across

landscapes has also been done in terrestrial mammal rabies research, particularly in raccoon rabies in the United States (Smith, Waller, Childs, & Real, 2002; Real, Russell, Waller, Smith, & Childs, 2005).

Evidence has also been found suggesting that seasonality plays a large role in vampire bat rabies expansion in Peru, specifically relating to host male dispersal patterns and births followed by parent to offspring vertical RABV transmission (Streicker et al., 2016; Benavides et al., 2016; Lord, 1992). This recent evidence is further supported by work confirming the effects of seasonality in raccoon rabies spread in the United States (Duke-Sylvester et al., 2011). In addition to seasonality, another notable element potentially influencing viral expansions and invasions in Peru is anthropogenic change, such as culling campaigns, land use, livestock density and usage, and deforestation (Streicker et al., 2012; Benavides et al., 2016; Schneider et al., 2009).

Public health implications

Previous rabies control efforts in Latin America have relied heavily on culling the bat populations (Benavides et al., 2016; Condori-Condori et al., 2013). However, this is a difficult method to implement successfully, since bats are airborne movers, which can hamper culling logistics, and due even more so to some emerging evidence suggesting that culling campaigns could unintentionally promote viral dispersal (Benavides et al., 2016; Streicker et al., 2012). While part of the elevated importance of zoonotic bat rabies is due to improved rabies surveillance, the ecological and anthropogenic changes must also be considered before simply attributing increasing cases to better disease detection (Condori-Condori et al., 2013; Streicker et al., 2012).

As a public health problem, rabies presents a significant mortality and economic burden within human and animal health, and has demonstrated biological persistence in its many viral variants across its respective reservoir species that enables potential spillover infections or secondary host adaptation (WHO, 2013; Real et al., 2005; Childs & Real, 2007). Given that livestock are dead-end hosts, there is no established livestock to human zoonotic transmission risk, however, there is concern that continued bat expansions and virus invasions across the landscape will cause an increase in both human and livestock rabies cases by increasing exposures due to proximity and bat population growth (Streicker et al., 2016, Benavides et al., 2016; Condori-Condori et al., 2013). Without adequate interventions, rabies expansions across Peru's landscape will serve to increase Peru's national rabies burden, exacerbate rabies risk in livestock health, and negatively impact agricultural contributions to the economy (Streicker et al., 2016; Benavides et al., 2016).

Goals & Questions

This research expanded upon the work of Streicker et al. (2016) and Benavides et al. (2016) in investigating the speed of vampire bat transmitted rabies spread across the landscape in Peru. The projects goals included: (i) identifying spatio-temporal clusters of outbreaks using national level livestock RABV incidence data (2003-2016), (ii) identifying the most likely outbreak origins of spatio-temporal clusters, and (iii) estimating the rate at which vampire bat RABV is moving across the landscape in identified outbreak clusters. Estimating rabies spread and identifying most likely

outbreak origins will aid creating more accurate predictions of future RABV invasions in Peru and other landscapes.

Methods

Data

The reported livestock rabies outbreak data were assembled by infectious disease ecologists Daniel Streicker and Julio Benavides at the University of Glasgow, and originated from publicly available national records from Peru's Servicio Nacional de Sanidad Agraria (SENASA). The outbreak reports (n=2992) cover national level suspected livestock vampire bat transmitted rabies outbreaks from 2003 to 2016 (Benavides et al., 2016). The reports include direct fluorescent antibody results from brain tissue samples, when possible, which confirm or refute reports of suspected outbreaks. The main variables of interest were rabies sample test results, time of reported outbreak, and reported latitude and longitude coordinates.

The outbreak data were separated into two subsets, one with the confirmed positive outbreaks, and the second with suspected outbreaks that designated all confirmed positive and negative outbreaks as suspected positives. The suspected subset was included due to questions as to the veracity of the confirmed laboratory results. As stated, the SENASA outbreaks brain tissue samples were tested using the direct fluorescent antibody test for rabies, which is the standard testing procedure (Brown et al., 2016). Many samples have to be shipped from rural areas of Peru to laboratory facilities, which allows for sample degradation and thus increases the likelihood of false negative results (Duong et al., 2016). The criteria for including outbreaks in either subset included:

necessary geographic information, time of the outbreak, and a positive or negative direct fluorescent antibody test result. Reported outbreaks missing geographic information or giving results other than negative or positive, such as sample not tested, were dropped. After dropping reports that did meet the criteria for inclusion, the confirmed outbreak subset included 1,374 positive outbreaks and the suspected outbreak subset included 2,364 outbreaks (Positive=1,374; Negative=990).

Analysis

Spatio-temporal clusters were identified using the SatScan software and scans for clusters were conducted using the space-time permutation model (Kulldorff, 2003). SatScan input case and coordinate files were created using the rsatscan (Kleinman, 2015) R package. The data were scanned separately twice, at the year, and year month levels. Both scan levels found the same clusters, with eight total confirmed outbreak clusters and thirteen total suspected outbreak clusters. The subsequent analysis used the year scan datasets.

The spatio-temporal clusters were analyzed with R software (R Core Team, 2016) to identify the most likely outbreak origin locations for each cluster. Simple linear regression models positioned against time were used to identify the likely outbreak origins and to estimate rates of spread in kilometers within each cluster. The simple linear regression models searched the area containing the minimum and maximum latitude and longitude coordinates of outbreaks. The regression criteria assumed that the most likely outbreak origin was located within the area of each cluster. Separate linear regressions were run for two time levels for both subsets: years and weeks. Each regression was run through 100,000 simulations using the minimum and maximum latitude and longitudes to

generate random coordinates and find the distance from the origins, using the "fields"

package (Nychka, Furrer, Paige, & Sain, 2015). Time was included by finding the

difference in time from the earliest outbreak within a cluster, successively running

through time of each outbreak minus the previous outbreak time. The simulations

identified the best fit r^2 value to infer the most likely outbreak origin of each cluster

(Streicker et al., 2016). The regression models were re-fit based on the best r^2 value, the

slope was used to generate the rate of RABV spread by cluster in kilometers, and each

cluster was plotted as a no-intercept regression, using both the outbreak origin and cluster

specific coordinates. The regression analysis most likely outbreak origins were visualized

in descriptive maps, using ESRI ArcMap software, alongside the outbreak cluster specific

point distributions (ESRI, 2015). The suspected outbreak clusters regression analysis at

the weekly level generated several high rates (Table 4). The outbreak clusters with

especially high rates were subset and re-run through SatScan individually, to determine if

a finer cluster scale would be identified. The subset dataset of clusters was then also

analyzed using simple linear regression to identify the most likely outbreak origins and

the rates of spread were estimated. To check for convergence of estimates, the regression

analyses on the weekly time scale for the confirmed and suspected cluster datasets were

run through five trials of the 100,000 regression simulations to verify consistency in the

most likely outbreak origin estimates. To determine if there was an association between

the rate of spread and the number of outbreaks, a correlation analysis test was performed

on all three datasets of clusters.

Results

The SENASA suspected outbreak dataset of clusters from SatScan is larger, with

a total of 734 outbreaks spread across 13 clusters, than the confirmed outbreak clusters

dataset from SatScan, with 379 outbreaks spread across 8 clusters (Figure 1a, 1b).

Clusters 2 and 6 in the confirmed outbreak data had the highest proportions of SENASA

outbreaks identified as part of a cluster (Table 1). In the suspected outbreak dataset,

clusters 7, 8, and 9 had the highest proportion of suspected SENASA outbreaks (Table 2).

The subset of suspected clusters (Figure 13a, 13b, 13c) output 202 outbreaks spread out

within 21 clusters, which broke up several of the clusters found in the first suspected

outbreak group of clusters (Figure 1b). The regression analyses at the weekly time scale

generated the finest scale r^2 and rate estimates (Table 3, 4, & 5).

Using the t-statistic, all regression results in both the confirmed and suspected

SENASA clusters were found to be statistically significant (alpha = $p < 0.05$) (Table 3 &

4). The suspected clusters subset regressions for clusters 13a and 13b (p=0.45; p=0.46)

were found not significant (Table 5). All other subset clusters were found to be

statistically significant (alpha = $p < 0.05$) (Table 5). The convergence trials for confirmed

outbreak and suspected outbreak SENASA subsets found consistent estimates of latitude

and longitude for the most likely outbreak origin within 2 decimal places, indicating that

the origin is consistently estimated by the regression analysis.

In the confirmed outbreak dataset, cluster 4 had the highest r^2 fit (r^2=0.84) at the

week scale (Table 3, Figure 6a). The estimated rate of spread for confirmed cluster 4 was

0.33 kilometers per week, and reported with 95% confidence that repeated trials will

generate a spread rate within 0.27-0.39 kilometers per week (Table 3). The confirmed

clusters 5 and 6 also had high r^2 values (Table 3, Figure 8a & 8b). In the suspected

SENASA dataset analysis, cluster 11 had the highest r^2 fit (r^2=0.84) (Table 4, Figure 6b)

and in the suspected dataset subset, clusters 9a and 9c had the highest r^2 values and the

best fit (r^2=0.98) (Table 5).

The regression analysis on the confirmed outbreak clusters at the week level

found estimated rates of spread ranging from 0.33 to 6.68 kilometers per week (17.17-

347.61 km/yr) (Table 3). The regression analysis run on suspected outbreak clusters at

the week level estimated rates of spread spanning from 0.22-5.03 kilometers per week

(11.54-261.52 km/yr) (Table 4). The regression analysis on the subset of the suspected

clusters estimated rates of spread ranging from 0.08 to 3.90 kilometers per week (4.22-

202.87 km/yr) (Table 5). The Pearson's product correlation on the confirmed clusters

between weekly rate of spread and the number of outbreaks was not significant (p=0.66)

and the point estimate was 0.18, suggesting a weak positive relationship. Given the small

sample size (n=8), the point estimate is evidence of a meaningful effect. The Pearson

product correlation found that the association between weekly rate of spread and the

number of outbreaks was not statistically significant, with a point estimate of 0.45,

suggesting a meaningful effect and a moderate positive relationship. The Pearson product

correlation on the subset of the original suspected clusters found that the association

between weekly rate of spread and the number of outbreaks was not statistically different

from 0 (p=0.97) and the point estimate of -0.009 found a weak negative relationship.

The confirmed outbreak clusters 2, 3, and 8 all displayed unique point

distributions and most likely origins (Figure 2b, Figure 3a, & Figure 5b). Confirmed

cluster 2 displayed a consistent spread across the cluster area, like a wave, (r^2=0.74).

(Figure 2b). Confirmed cluster 3 displayed a distinct east-west band, likely originating from the south (Figure 3a). Confirmed cluster 8 visualized a north-south band wave, likely originating in middle of the cluster area (Figure 5b). The suspected outbreak clusters 4, 5, 6, 8, 9, and 12 also visualize unique point patterns (Figure 7b, 8a, 8b, 10a, & 11b). Suspected cluster 8 displayed consistent RABV within the cluster area and cluster 4 visualized three near distinct spots within the cluster area (Figure 7b & 9b). Suspected cluster 4 is further broken into five subset clusters, which could indicate multiple waves present within the cluster 4 area (Figure 7b & 13b). Suspected cluster 5 shows two large concentration regions of outbreaks, with the most likely origin from the weekly regression analysis indicating west to east spread in a wave (r^2=0.70) (Figure 8a, Table 4).

The suspected cluster weekly regression scale analysis found r^2 values at or above 0.80 for clusters 6, 7, 11, and 13, which indicates reasonably good r^2 fits for the no-intercept regression lines (Table 5). The suspected clusters 6, 7, and 8 outbreak distributions and the most likely origins suggested potentially endemic RABV areas (Figure 8b, 9a, & 9b). Suspected cluster 9 also potentially suggested RABV is endemic, considering the distinct and dense line patterns and the three-year cluster interval, indicating RABV has remained in the bat populations (Figure 10a). The suspected cluster 13 distribution pointed to a north to NE spread pattern (Figure 12a).

Discussion

Several spatio-temporal clusters were identified across the landscape and study period (2003-2016), with minimum estimated rates of spread of 17.17 (or 11.54)

kilometers per year (0.33-6.68 km/wk; 0.22-5.03 km/wk) and indicated the presence of consistent RABV waves of spread across the Peruvian landscape within the 14 year study period (Table 3, 4, & 5). This conclusion is supported by the recent findings of Benavides et al. (2016), which found wavefront RABV spread in areas of Peru within a smaller time period. No statistically significant associations were found in cluster datasets between rates of spread and number of outbreaks, however meaningful effects of point estimates were observed. All three outbreak cluster datasets demonstrated moderate to high range in the best r^2 and the subsequent rate estimates. Despite the difference in total clusters between datasets, the ranges of spread per week are very similar. All of the confirmed and suspected outbreaks regression models were found to be statistically significant, however, as the regression models were plotted with no-intercept, positive slopes are more likely to occur as the regression line is forced through the origin.

The study's main strength was using fourteen years of national scale georeferenced outbreak data with sample results. The SENASA data is a considerably robust dataset by rabies research standards, especially when the rabies variant in question is transmitted by the very complex vampire bat host-reservoir. There was moderate to high variability in the cluster regression analyses r^2 values, with several values of medium fit (Table 3, 4, & 5). The variability indicates that the linear regression criteria is a potential limitation to the analysis. The regression model criteria assumed that the most likely outbreak origin occurred within the area of the identified outbreak cluster, which limited the bounds of the results. Additionally, in the cases of larger clusters, the variability in the r^2 fits could be attributed to the presence of multiple wave patterns of RABV spread, which could be masked by the SatScan cluster grouping. The variability could also be indicating a bias in

cluster grouping via potential correlation between time and the most likely outbreak origins. The variability illustrates a need for further analysis to draw definitive conclusions.

An important second limitation was the uncertainty associated with the rabies tissue sample test results due to questionable test conditions that may have promoted false positive results in the direct florescent antibody test. However, this was at least in part addressed by the inclusion of a suspected dataset that treated all confirmed positive and negative results as positive outbreaks. While the suspected group analysis results must be considered conservatively, it does provide plausible enough data for finding RABV wavefront spread patterns across the landscape, given that all outbreaks, regardless of testing results were reported as potential disease cases. Outbreak reporting is the final main limitation, as the surveillance data collected by SENASA is prompted by reports of livestock disease, and Peru is known to have under-reporting of rabies (WHO, 2013). This is likely especially true in rural areas where most livestock cases are found, because ownership is associated with farms (WHO, 2013).

Building on this work, further analysis should assess bias in clustering via testing for correlating relationships between rates and geographic information and time period. Convex hull polygons could be created to visualize the outbreak points and enable area calculations for running correlation analysis. Follow-up investigation should pursue landscape factors, such as livestock density and elevation, to determine if there is an association between the landscape factors and the estimated rates of spread in the identified spatio-temporal clusters.

This research, in conjunction with the suggested further analysis could aid in implementing RABV control measures in Peru to prevent both livestock and spillover human infections. A great deal of past efforts and funding have attempted to control vampire bat transmitted RABV in Peru, and throughout Latin America, using culling campaigns when high incidence of RABV prompted response (Benavides et al., 2016; Condori-Condori et al., 2013; Stoner-Duncan et al., 2014). Culling campaigns are increasingly considered not effective enough for adequate control of RABV spread, however the literature presents alternatives such as vampire bat vaccination campaigns and promoting research into non-lethal and sustainable methods of bat population control (Almeida, Martorelli, Aires, Barros, & Massad, 2008; Stoner-Duncan et al., 2014). Regardless of the future methods employed in vampire bat transmitted RABV control, this research can provide targeted spatial identification and forecasting for the implementation of control measures. The identified spatio-temporal clusters, wavefront spread, and estimated rates of spread per week could be used to apply preventative vaccinations, in livestock, humans, or bats, in areas determined at risk to an approaching wavefront. The research could also potentially be used to hypothesize vampire bat roosting sites. Lastly, targeted research could use the identified spatio-temporal clusters to sample livestock at farms within the cluster area, to investigate area prevalence in relation to reported incidence cases.

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Tables & Figures

Table 1. Proportions of confirmed outbreaks found by cluster

Cluster	Confirmed % (N= 1,374)
1	1.6
2	4.4
3	1.0
4	6.7
5	4.1
6	3.8
7	1.9
8	4.1

Table 2. Proportions of suspected outbreaks found by cluster

Cluster	Suspected % (N= 2,364)
1	1.5
2	3.9
3	0.4
4	2.2
5	0.5
6	2.0
7	4.3
8	6.0
9	4.6
10	1.2
11	1.6
12	1.9
13	1.0

Table 3. Confirmed outbreak clusters regression analysis (weekly time scale)

Cluster Number	Outbreaks (N=)	Start Time	End Time	R^2	Weekly rate (km/wk)	*C.I. (95%)	Annual rate (km/yr)	p-value
7	22	2003	2004	0.66	0.54	(0.36-0.71)	27.90	<.001
1	60	2003	2005	0.70	6.68	(5.56-7.81)	347.61	<.001
6	14	2005	2006	0.80	1.64	(1.14-2.13)	85.16	<.001
3	92	2006	2008	0.66	0.66	(0.56-0.75)	34.10	<.001
2	57	2009	2011	0.58	0.49	(0.38-0.60)	25.48	<.001
5	52	2013	2014	0.80	1.25	(1.07-1.43)	64.99	<.001
4	26	2014	2014	0.84	0.33	(0.27-0.39)	17.17	<.001
8	56	2015	2016	0.72	2.37	(1.97-2.78)	123.47	<.001

*95% confidence interval calculated for the weekly rates

Table 4. Suspected outbreak clusters regression analysis (weekly time scale)

Cluster number	Outbreaks (N=)	Start Time	End Time	R^2	Weekly rate (km/wk)	*C.I. (95%)	Annual rate (km/yr)	p-value
5	36	2003	2004	0.70	0.65	(0.50-0.79)	33.76	<.001
2	92	2003	2006	0.74	5.03	(4.41-5.65)	261.52	<.001
11	9	2004	2004	0.84	0.29	(0.19-0.40)	15.34	<.001
12	52	2004	2006	0.67	4.77	(3.83-5.71)	248.03	<.001
13	13	2004	2005	0.83	2.91	(2.09-3.74)	151.43	<.001
8	47	2007	2008	0.68	0.63	(0.50-0.75)	32.63	<.001
1	101	2009	2011	0.60	0.39	(0.33-0.45)	20.34	<.001
9	141	2012	2015	0.70	3.69	(3.29-4.10)	192.10	<.001
4	109	2013	2014	0.77	2.91	(2.61-3.21)	151.24	<.001
6	29	2014	2014	0.83	0.29	(0.24-0.34)	15.15	<.001
7	38	2015	2016	0.82	0.31	(0.27-0.36)	16.32	<.001
3	44	2016	2016	0.60	0.22	(0.17-0.28)	11.54	<.001
10	23	2016	2016	0.72	1.84	(1.33-2.35)	95.93	<.001

*95% confidence interval calculated for the weekly rates

Table 5. Suspected outbreak clusters subset regression analysis (weekly time scale)

Cluster number	Outbreaks (N=)	Start Time	End Time	R^2	Weekly rate (km/wk)	*C.I. (95%)	Annual rate (km/yr)	p-value
2c	13	2003	2003	0.58	0.39	(0.18-0.60)	20.36	<.001
2d	11	2004	2004	0.58	0.40	(0.18-0.62)	20.68	<.001
12e	7	2004	2004	0.46	2.64	(-0.23-5.51)	137.25	<.05
12c	7	2004	2004	0.51	3.08	(0.07-6.09)	160.07	<.05
13a	3	2004	2004	0.35	0.22	(-0.68-1.11)	11.27	0.45
2b	6	2004	2004	0.92	0.23	(0.15-0.30)	11.73	<.05
2a	21	2005	2006	0.65	3.12	(2.04-4.21)	162.50	<.001
12a	5	2005	2005	0.83	1.16	(0.44-1.88)	60.29	<.05
13b	2	2005	2005	1.00	0.80	(0.80-0.80)	41.81	0.46
12b	11	2006	2006	0.73	1.54	(0.89-2.19)	80.03	<.001
12d	4	2006	2006	0.77	0.78	(0.0-1.56)	40.43	<.05
9c	9	2012	2012	0.98	0.10	(0.09-0.11)	5.36	<.05
4e	5	2013	2013	0.88	0.27	(0.13-0.41)	14.06	<0.05
4a	27	2013	2013	0.82	3.90	(3.16-4.65)	202.87	<.001
4d	8	2013	2013	0.94	0.16	(0.12-0.20)	8.40	<.001
9d	5	2013	2013	0.97	0.15	(0.12-0.19)	7.95	**
4b	7	2014	2014	0.50	0.40	(0.0-0.81)	21.01	<.05
4c	9	2014	2014	0.83	0.56	(0.35-0.76)	28.96	<.001
9a	13	2014	2014	0.98	0.08	(0.07-0.09)	4.22	<.001
9b	26	2015	2015	0.53	1.13	(0.69-1.57)	58.84	<.001

*95% confidence interval calculated for the weekly rates

**T-statistic did not calculate
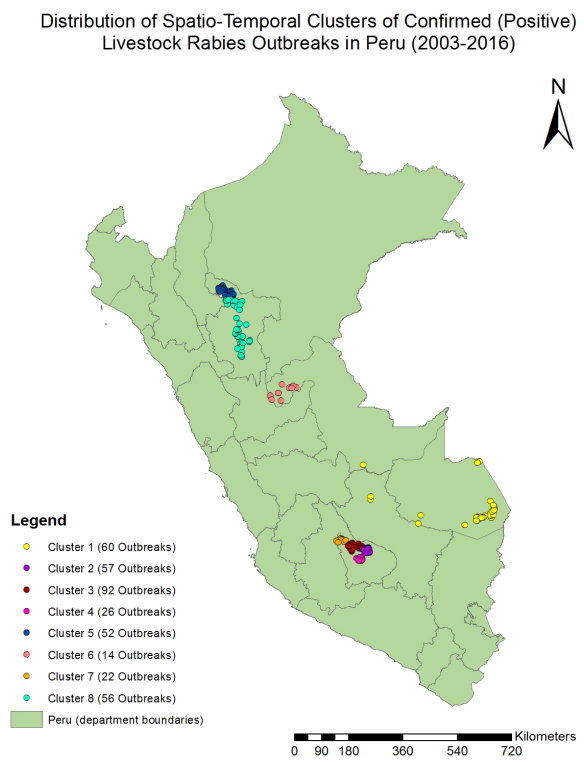
Figure 1. Outbreak cluster distributions in Peru

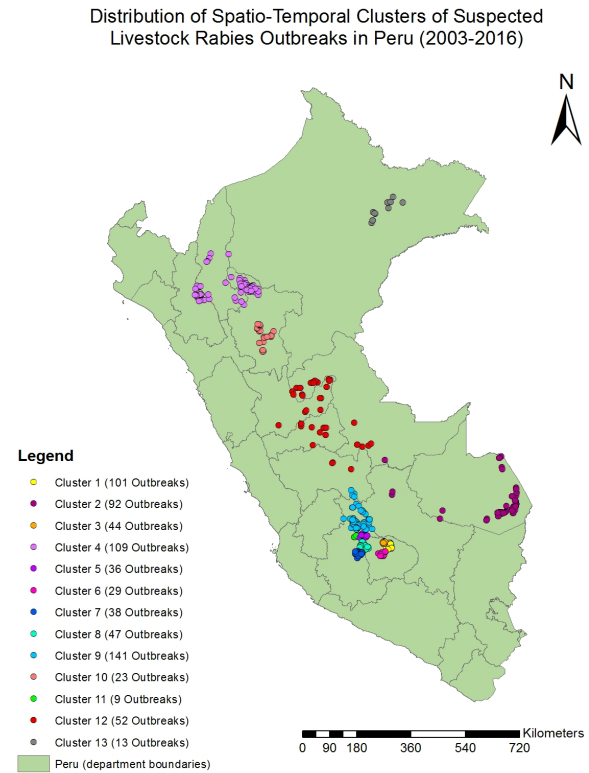Distribution of Spatio-Temporal Clusters of Confirmed (Positive)
Livestock Rabies Outbreaks in Peru (2003-2016)

Legend

- Cluster 1 (60 Outbreaks)
- Cluster 2 (57 Outbreaks)
- Cluster 3 (92 Outbreaks)
- Cluster 4 (26 Outbreaks)
- Cluster 5 (52 Outbreaks)
- Cluster 6 (14 Outbreaks)
- Cluster 7 (22 Outbreaks)
- Cluster 8 (56 Outbreaks)
- Peru (department boundaries)

Figure 1(a)

Distribution of Spatio-Temporal Clusters of Suspected
Livestock Rabies Outbreaks in Peru (2003-2016)

Legend

- Cluster 1 (101 Outbreaks)
- Cluster 2 (92 Outbreaks)
- Cluster 3 (44 Outbreaks)
- Cluster 4 (109 Outbreaks)
- Cluster 5 (36 Outbreaks)
- Cluster 6 (29 Outbreaks)
- Cluster 7 (38 Outbreaks)
- Cluster 8 (47 Outbreaks)
- Cluster 9 (141 Outbreaks)
- Cluster 10 (23 Outbreaks)
- Cluster 11 (9 Outbreaks)
- Cluster 12 (52 Outbreaks)
- Cluster 13 (13 Outbreaks)
- Peru (department boundaries)

Figure 1 (b)

Figure 2. Confirmed outbreak clusters 1 and 2 linear regression analysis identified outbreak origins
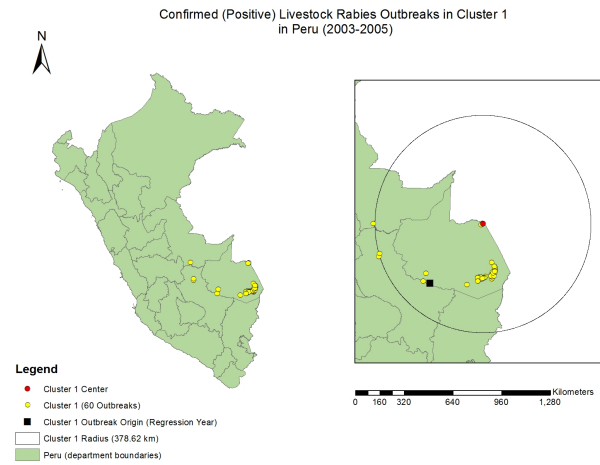
Figure 2 (a)
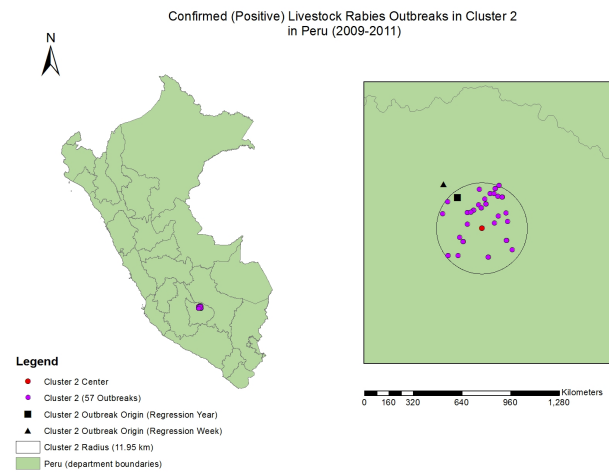
Figure 2 (b)

Figure 3. Confirmed outbreak clusters 3 & 4, linear regression analysis identified outbreak origins
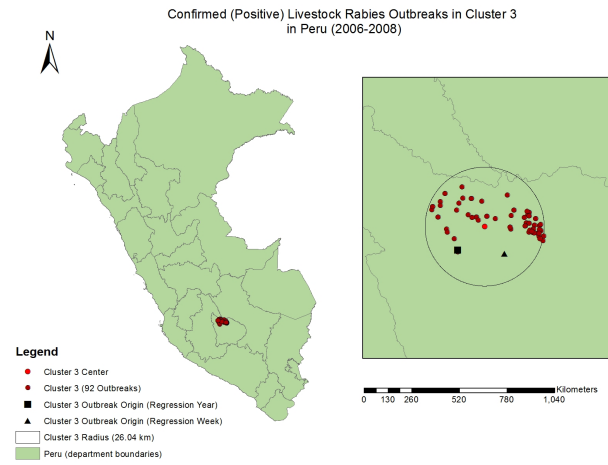
Figure 3 (a)
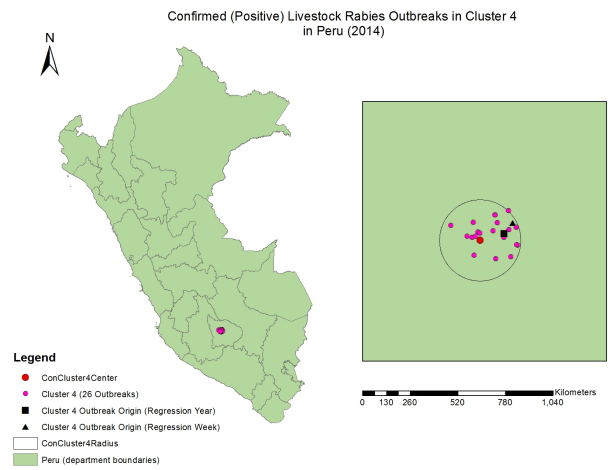
Figure 3 (b)

Figure 4. Confirmed outbreak clusters 5 & 6, linear regression analysis identified outbreak clusters
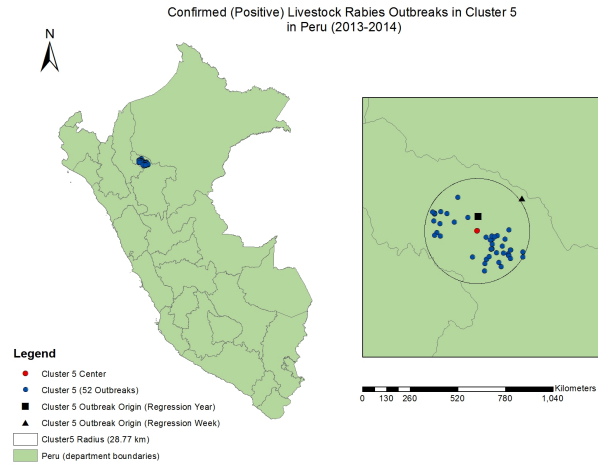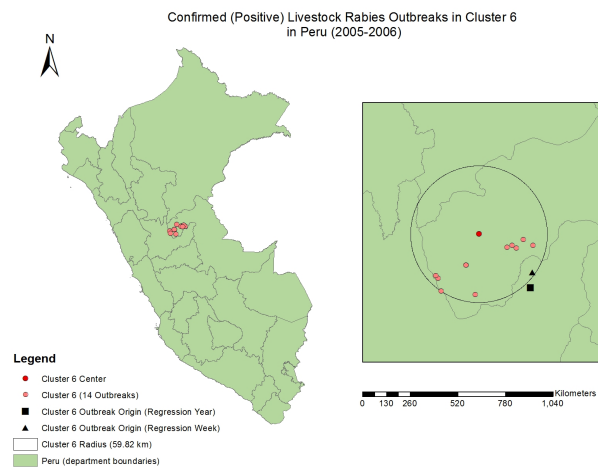

Confirmed (Positive) Livestock Rabies Outbreaks in Cluster 5
in Peru (2013-2014)

Legend
- Cluster 5 Center
- Cluster 5 (52 Outbreaks)
- Cluster 5 Outbreak Origin (Regression Year)
- Cluster 5 Outbreak Origin (Regression Week)
- Cluster5 Radius (28.77 km)
- Peru (department boundaries)

Figure 4 (a)

Confirmed (Positive) Livestock Rabies Outbreaks in Cluster 6
in Peru (2005-2006)

Legend
- Cluster 6 Center
- Cluster 6 (14 Outbreaks)
- Cluster 6 Outbreak Origin (Regression Year)
- Cluster 6 Outbreak Origin (Regression Week)
- Cluster 6 Radius (59.82 km)
- Peru (department boundaries)

Figure 4 (b)

Figure 5. Confirmed outbreak clusters 7 & 8, linear regression analysis identified outbreak origins
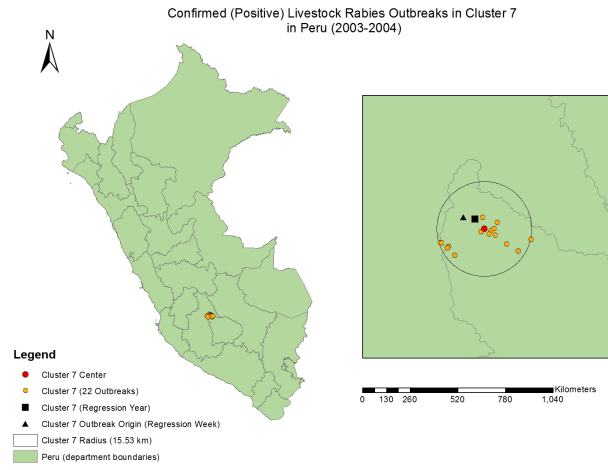
Figure 5 (a)
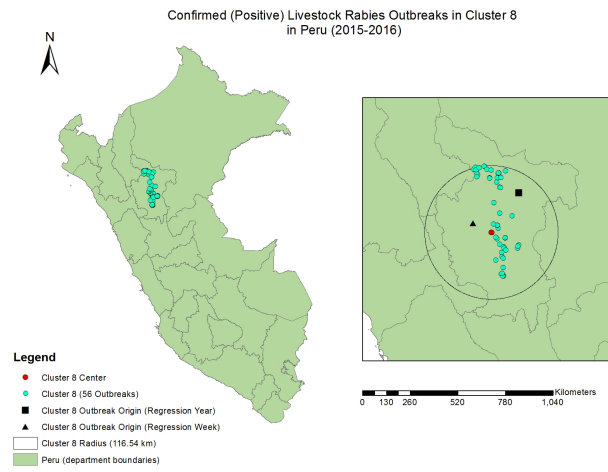
Figure 5 (b)

Figure 6. Suspected outbreak clusters 1 & 2, linear regression analysis identified outbreak origins
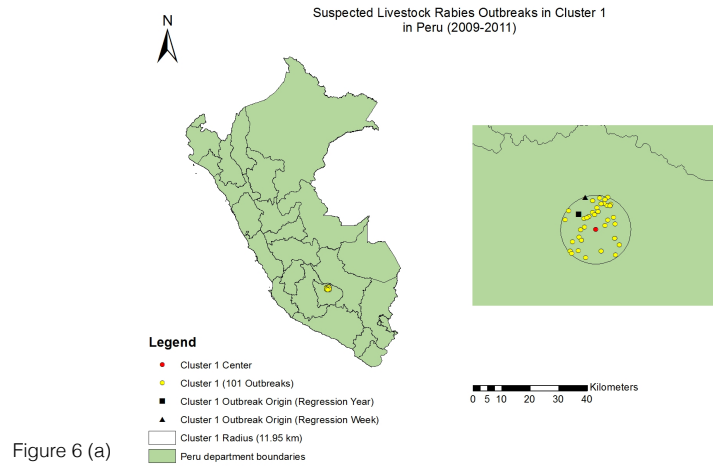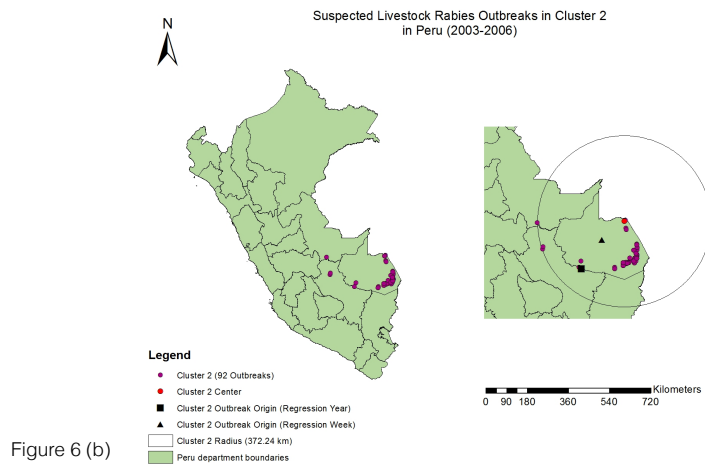
Suspected Livestock Rabies Outbreaks in Cluster 1
in Peru (2009-2011)

N

Legend
● Cluster 1 Center
● Cluster 1 (101 Outbreaks)
■ Cluster 1 Outbreak Origin (Regression Year)
▲ Cluster 1 Outbreak Origin (Regression Week)
☐ Cluster 1 Radius (11.95 km)
Peru department boundaries

Kilometers
0 5 10   20   30   40

Figure 6 (a)

Suspected Livestock Rabies Outbreaks in Cluster 2
in Peru (2003-2006)

N

Legend
● Cluster 2 (92 Outbreaks)
● Cluster 2 Center
■ Cluster 2 Outbreak Origin (Regression Year)
▲ Cluster 2 Outbreak Origin (Regression Week)
☐ Cluster 2 Radius (372.24 km)
Peru department boundaries

Kilometers
0 90 180   360   540   720

Figure 6 (b)

Figure 7. Suspect outbreak clusters 3 & 4, linear regression analysis identified outbreak origins
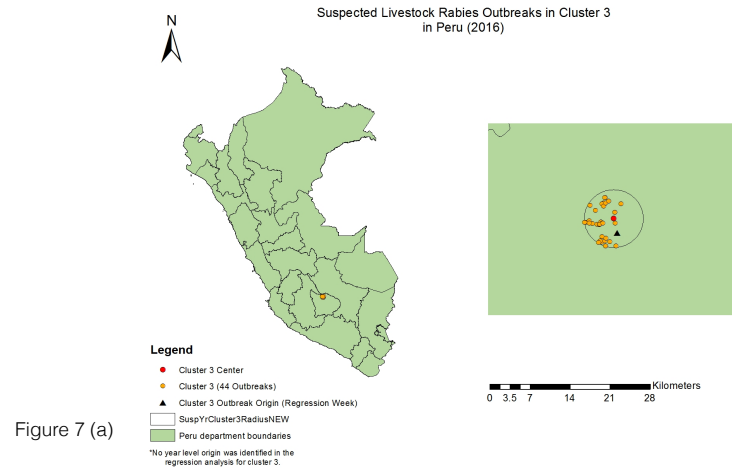
Figure 7 (a)
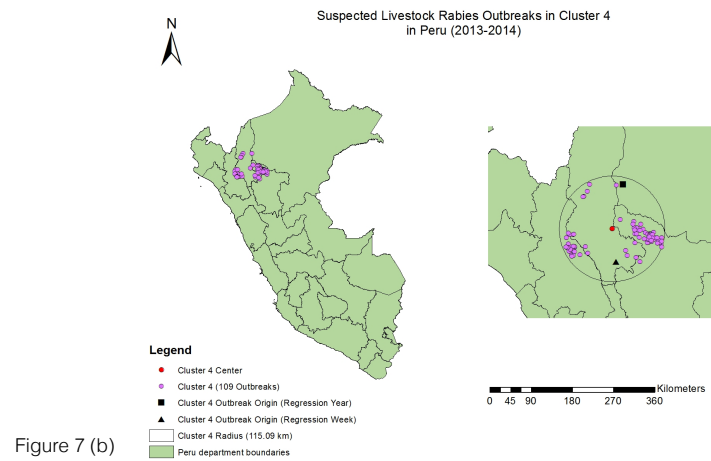
Figure 7 (b)

Figure 8. Suspected outbreak clusters 5 & 6, linear regression analysis outbreak origins
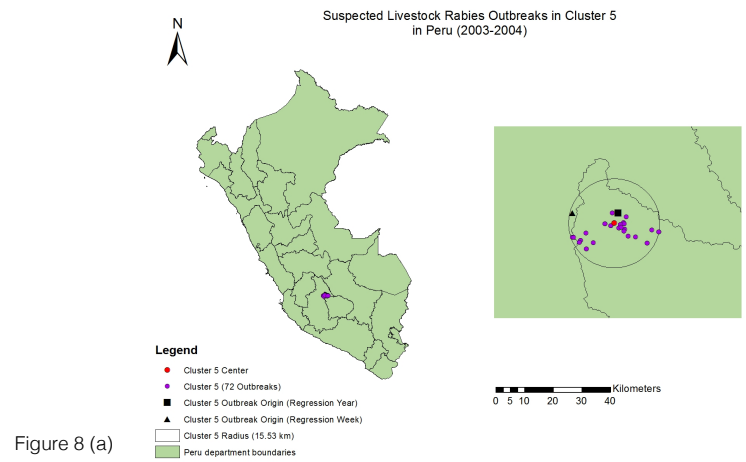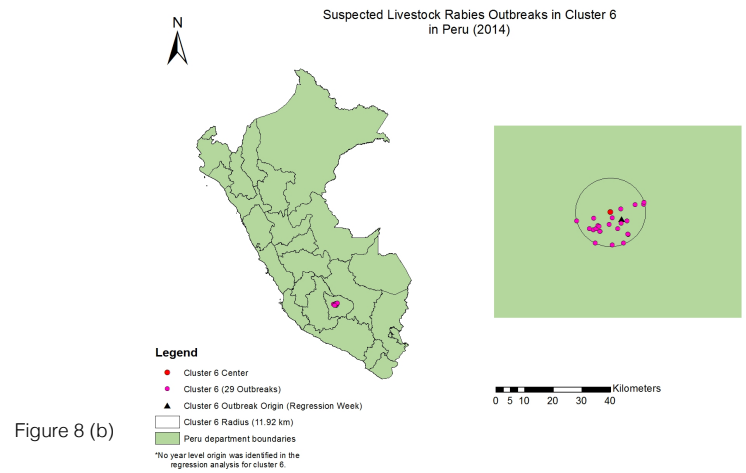

Figure 8 (a)

Figure 8 (b)

Figure 9. Suspected outbreak clusters 7 & 8, linear regression analysis outbreak origins
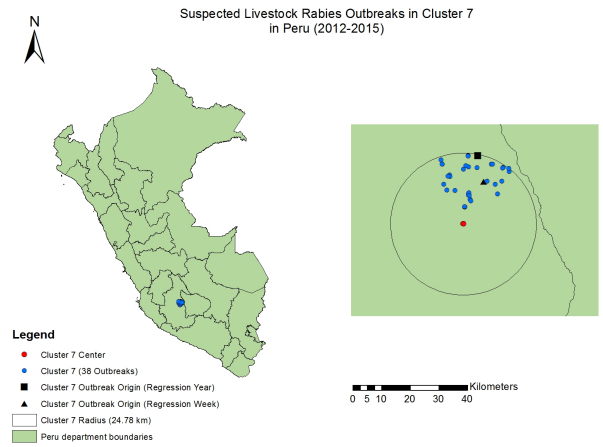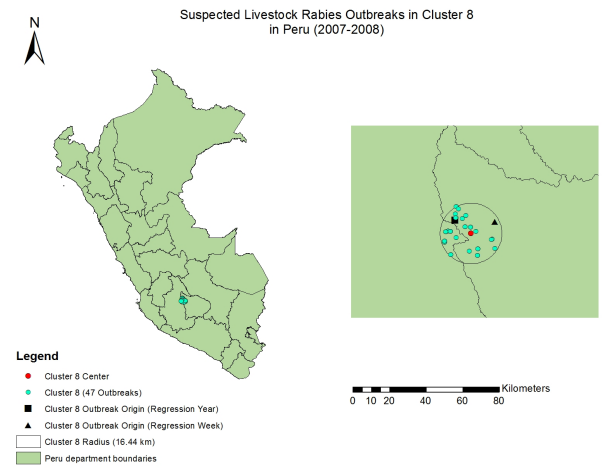

Figure 9 (a)

Figure 9 (b)

Figure 10. Suspected outbreak clusters 9 & 10, linear regression analysis outbreak origins
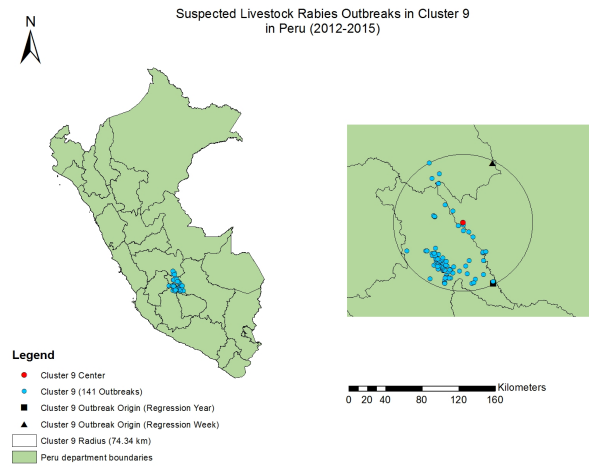
Suspected Livestock Rabies Outbreaks in Cluster 9
in Peru (2012-2015)

Legend
● Cluster 9 Center
● Cluster 9 (141 Outbreaks)
■ Cluster 9 Outbreak Origin (Regression Year)
▲ Cluster 9 Outbreak Origin (Regression Week)
▢ Cluster 9 Radius (74.34 km)
▨ Peru department boundaries

Kilometers
0 20 40   80   120   160

Figure 10 (a)

Suspected Livestock Rabies Outbreaks in Cluster 10
in Peru (2016)

Legend
● Cluster 10 Center
● Cluster 10 (23 Outbreaks)
▲ Cluster 10 Outbreak Origin (Regression Week)
▢ Cluster 10 Radius (59.82 km)
▨ Peru department boundaries

*No year level origin was identified in the
regression analysis for cluster 10.

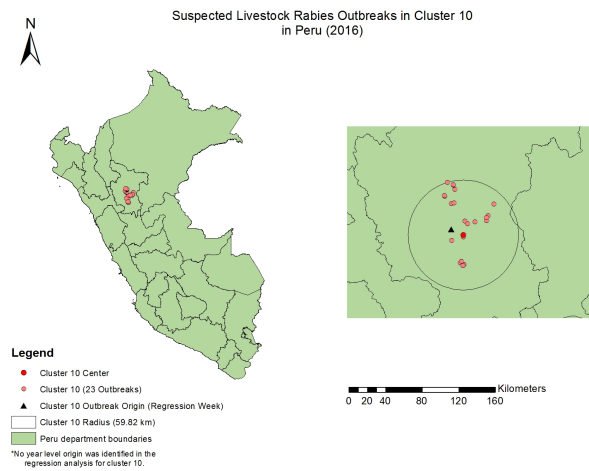Kilometers
0 20 40   80   120   160

Figure 10 (b)

Figure 11. Suspected outbreak clusters 11 & 12, linear regression analysis outbreak origins

Figure 11 (a)

Figure 11 (b)

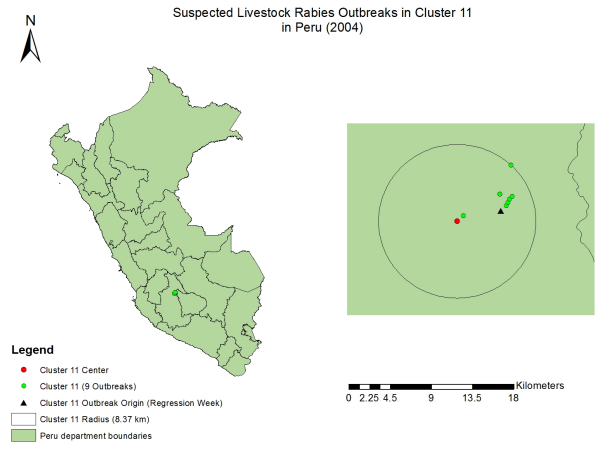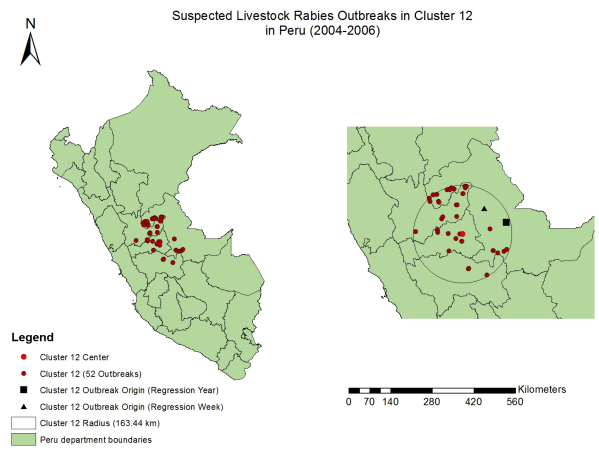Figure 12. Suspected outbreak cluster 13, linear regression analysis outbreak origins
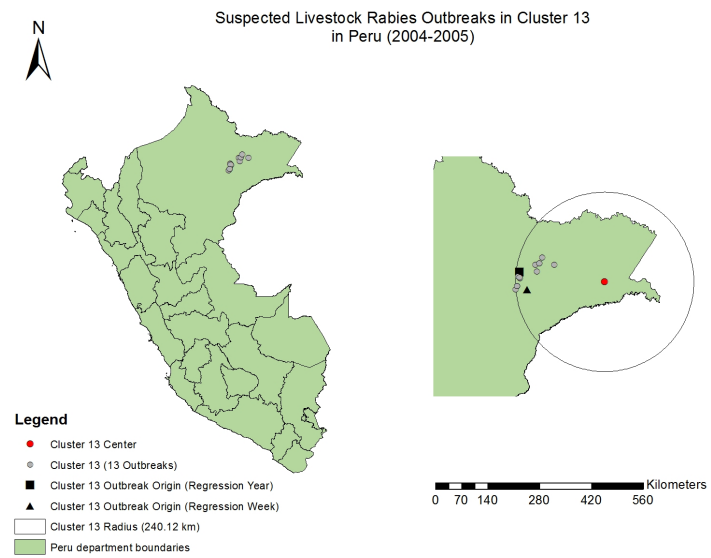

Suspected Livestock Rabies Outbreaks in Cluster 13
in Peru (2004-2005)

N

Legend
- ● Cluster 13 Center
- ● Cluster 13 (13 Outbreaks)
- ■ Cluster 13 Outbreak Origin (Regression Year)
- ▲ Cluster 13 Outbreak Origin (Regression Week)
- Cluster 13 Radius (240.12 km)
- Peru department boundaries

Kilometers
0   70 140   280   420   560

Figure 13. Subset of smaller suspected clusters 2, 4, 9, 10, 12, & 13
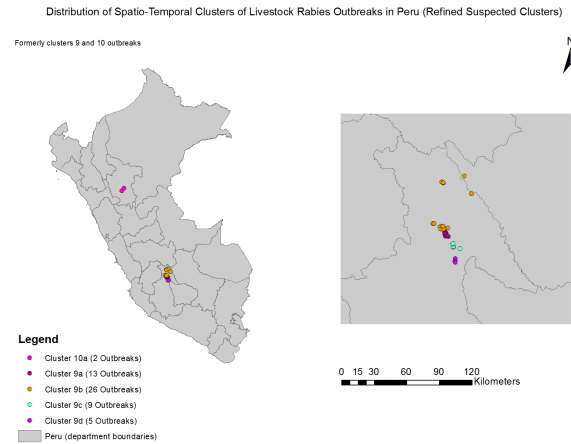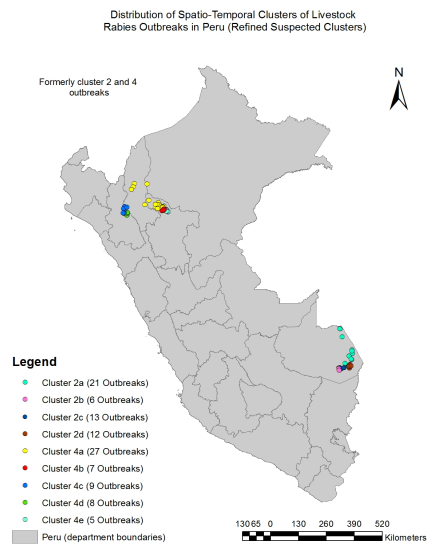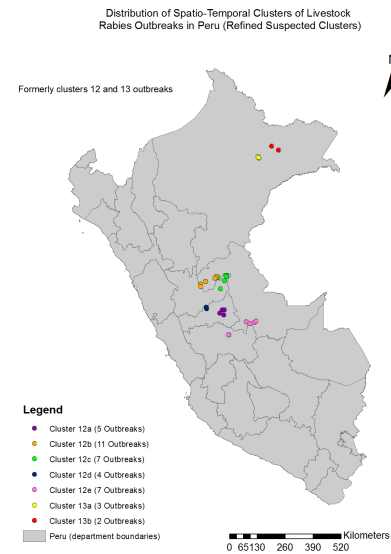

Figure 13 (a)

Figure 13 (b)

Figure 13 (c)

Figure 14. Cluster outbreaks by weeks and stance from most likely outbreak origins

Confirmed Cluster 4 (By Week) Scatterplot

(a)

Suspected Cluster 11 (By Week) Scatterplot

(b)

Suspected Refined Cluster 9a (By Week) Scatterplot

(c)