Characterization of the epidemiology of bat-borne rabies in Chile between 2003 and 2013

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\textbf{A B S T R A C T}

Rabies is a zoonotic disease of great impact to public health. According to the World Health Organization, the country of Chile is currently declared free from human rabies transmitted by dogs. An epidemiological characterization and description was conducted using rabies data from 2003 to 2013 held by the National Program for Prevention and Control of Rabies from the Ministry of Health, consisting of bats samples reported as suspect and samples taken by active surveillance (bats brain tissue). Spatial autocorrelation analysis was performed using Local Indicators of Spatial Association (LISA) statistics, particularly Moran's I index, for the detection of spatial clusters. Temporal descriptive analysis was also carried out. Nine hundred and twenty-seven positive cases were reported, presenting an average of 84 cases per year, mainly originated from passive surveillance (98.5%); whilst only 1.5% of cases were reported by active surveillance. Global positivity for the study period was 7.02% and 0.1% in passive and active surveillance respectively. Most of the cases were reported in the central zone of Chile (81.1%), followed by south zone (9.1%) and north zone (2.8%). At a regional level, Metropolitana (40.6%), Valparaíso (19.1%) and Maule (11.8%) regions reported the majority of the cases. \textit{Tadarida brasiliensis} (92%) presented the majority of the cases reported, with viral variant 4 (82%) being most commonly diagnosed. Only two cases were detected in companion animals. The central zone presented a positive spatial autocorrelation (Moran’s I index = 0.1537, 95% CI = 0.1141–0.1933; p-value = 0.02); north and south zones returned non-significant results (Moran’s I index = 0.0517 and −0.0117, 95% CI = −0.0358–0.1392 and −0.0780–0.0546, and p-values = 0.21 and 0.34 respectively). The number of rabies cases decreased between May and August (late fall and winter) and tended to increase during the hot season (December to March), confirmed with the evidence from Autocorrelation analysis and the Ljun-Box test ($X^2 = 234.85$ and p-value < 0.0001). Knowledge of animal rabies epidemiologic behaviour becomes relevant when designing prevention and control measures and surveillance programs. This is especially important considering the high impact to Public Health of this disease and that wildlife rabies in bats remains endemic in Chile.

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1. Introduction

Rabies is a zoonotic disease caused by an RNA virus from the family Rhabdoviridae, genus \textit{Lyssavirus}, which has a case fatality rate in humans of near 100% if prophylactic measures are not implemented within a short time following exposure (Hampson et al., 2008; Dubovi and MacLachlan, 2011). Worldwide, rabies causes around 60,000 human deaths annually, 95% of which are from rural areas from Asia and Africa (WHO, 2016). In endemic regions, this disease is of major public health importance, not only due to the high case fatality rate, but also because it is potentially preventable if health education and immunization approaches are used. Due to the presence of the virus in both humans and animal hosts (with human infection almost invariably due to contact with infected animals) and its severity, control of rabies has been considered to be..
Table 1
Total number of samples and positivity percentage per year, from active and passive surveillance, of animal rabies in Chile, between the years 2003 and 2013.

<table>
<thead>
<tr>
<th>Year</th>
<th>Active surveillance</th>
<th>Passive surveillance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples</td>
<td>Positivity (%)</td>
</tr>
<tr>
<td>2003</td>
<td>1,657</td>
<td>1 (0.06)</td>
</tr>
<tr>
<td>2004</td>
<td>1,966</td>
<td>5 (0.25)</td>
</tr>
<tr>
<td>2005</td>
<td>1,840</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>2006</td>
<td>1,528</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>2007</td>
<td>1,781</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>2008</td>
<td>1,571</td>
<td>3 (0.19)</td>
</tr>
<tr>
<td>2009</td>
<td>810</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>2010</td>
<td>977</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td>2011</td>
<td>758</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>2012</td>
<td>418</td>
<td>2 (0.47)</td>
</tr>
<tr>
<td>2013</td>
<td>500</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>13,806</strong></td>
<td><strong>14 (0.10)</strong></td>
</tr>
</tbody>
</table>

Table 2
Number of cases of animal rabies in Chile, by animal reservoir and species, between the years 2003 and 2013.

<table>
<thead>
<tr>
<th>Animal reservoir</th>
<th>Species</th>
<th>N of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bat</td>
<td>T. brasiliensis</td>
<td>856</td>
</tr>
<tr>
<td></td>
<td>Lasius cinereus</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Histiotus macrotus</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Lasius borealis</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Myotis chilensis</td>
<td>9</td>
</tr>
<tr>
<td>Dog</td>
<td>Canis familiaris</td>
<td>1</td>
</tr>
<tr>
<td>Cat</td>
<td>Felis catus</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>927</strong></td>
</tr>
</tbody>
</table>

Table 3
Number of cases of animal rabies in Chile, by rabies virus variants detected in positive bats between the years 2008 and 2013.

<table>
<thead>
<tr>
<th>Viral variants detected on bats</th>
<th>N of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>381</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>H. macrotus</td>
<td>4</td>
</tr>
<tr>
<td>Not typified</td>
<td>46</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>465</strong></td>
</tr>
</tbody>
</table>

Table 4
Moran’s I index, 95% confidence intervals and p-value for the three macro-zones.

<table>
<thead>
<tr>
<th>Zone</th>
<th>Moran’s I</th>
<th>Confidence interval (95%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lower</td>
<td>upper</td>
<td></td>
</tr>
<tr>
<td>North</td>
<td>0.0517</td>
<td>-0.0358</td>
<td>0.1392</td>
</tr>
<tr>
<td>Central</td>
<td>0.1537</td>
<td>0.1141</td>
<td>0.1933</td>
</tr>
<tr>
<td>South</td>
<td>-0.0117</td>
<td>-0.078</td>
<td>0.0546</td>
</tr>
</tbody>
</table>

2. Materials and methods

2.1. Source population

For this study, secondary data sources were used, corresponding to the archives of the National Program for Prevention and Control of Rabies from MINSAL. In the case of active surveillance samples correspond to bats sampled directly from their colonies by MINSAL staff or other known reservoirs related to human bites (mainly dogs), in the case of passive surveillance, samples correspond to public reports of suspicious bats presence. This data source contained details of all positive cases, defined as brain samples positive to direct immunofluorescence (DI), from sampled animals, analysed by the Instituto de Salud Pública de Chile (ISP; Chilean Institute of Public Health), national reference laboratory in the diagnosis of rabies (ISP, 2013).

2.2. Target population

From these records, all available data was collected from positive cases between 2003 and 2013 and then consolidated recording: official registration number of the case, date of diagnosis, region and commune of occurrence, positive animal reservoir (dog, cat or bat), origin of the sample (active or passive surveillance) and the viral variant, the latter being registered since 2008. In terms of a definition of commune, there is no official definition in terms of dimensions or population ranges. This because there are 346 communes in Chile and with substantial differences between them. Normally the idea of a commune corresponds to a territory around an important urban nucleus, but not always happens in this way. There are communes of mostly rural characteristics without any defined urban nucleus, on the other side we have large conurbations divided in multiple communes, e.g. the city of Santiago de Chile, that have 36 communes. When describing communes in Chile by surface, it can be observed mean of 5,795.4 km², with ranges of 7.0 km²−49,924.1 km² (Indepencia and Natales surface respectively); by population, it can be observed a mean of 49,850 inhabitants, with ranges of 332 inhabitants to 805,000 inhabitants (Ollagüe and Maipú population respectively); and by human population density (measure in inhabitants per km²), it can be observed a mean of 862.62 inhabitants per km², with ranges of from 0.02 inhabitants per km² to 14,218.14 inhabitants per km² (Rio Verde and Lo Espejo population density respectively) (INE, 2017).
2.3. Data analysis

2.3.1. Descriptive analysis

Epidemiological characterization was performed using descriptive statistics; mean and proportion of cases of animal rabies reported, sample origin and cases per region were calculated. Animal reservoirs and viral variant were summarized by species and region involved.

2.3.2. Spatial and temporal analysis

Spatial mapping and analysis of the numbers of positive cases was conducted, using the commune, minor and basic administrative division in Chile, as the unit of interest (Pfeiffer et al., 2008). Because of the amount of information and the geographic characteristics of Chile three macro-zones were established: North, composed by the regions of Arica y Parinacota, Tarapacá, Antofagasta, Atacama and Coquimbo; Central, composed by the regions of Valparaíso, Metropolitana, O’Higgins, Maule and Biobio; and South, composed by the regions of Araucanía, Los Lagos, Los Rios, Aysén and Magallanes. The communes of Easter Island and Juan Fernández were not included in the current study, due to their particular geographical situation as these communes are considered insular territory free of rabies. Shapefiles of Chile and commune boundaries were obtained from Military Geographic Institute (IGM, from its Spanish acronym), and choropleth maps of the total number of cases of animal rabies over the study period were generated. Spatial investigation of potential clusters of disease, spatial autocorrelation (SA) within each macro-zone was conducted, consisting in a property of spatial data that is represented by an index that quantifies global dependence by measuring the correlation between observation of the same variable at different positions in the space, using Local Indicators of Spatial Association (LISA), with these indicators it is possible to verify how much contributes each case of animal rabies reported by commune to the formation of the general value, obtaining the degree of spatial association of the cases detected or the heterogeneity that result from the contribution of each spatial unit (Pfeiffer et al., 2008). The indicator used was the Moran’s I index, which establishes the degree of similarity between the number of reported cases of animal rabies in any area and that in the neighbouring areas, always associated with a hypothesis test, which is presented under the assumption of normality, through the presentation of a null hypothesis (H0) that establishes that there is no SA (Pfeiffer and Stevens, 2015), in our case these areas correspond to the communes. Three possible results can be obtained from the Moran’s I index, clustering of spatial units, in other words, neighbouring units have close values (positive SA); dispersion of the spatial units, neighbouring unit present very dissimilar values (negative SA); and none of the above situations occur, the values of neighbouring units are randomly produced (no SA) (Clements and Pfeiffer, 2009). We used descriptive statistics to identify potential temporal trends and seasonality in the registered date of diagnosis. Time series analysis was used by additive decomposition, to confirm the descriptive result, first estimating the three components of a seasonal time series, the trend component, the underlying trend of the measure, referred to long-term increase or decrease in the data; the seasonal component, the pattern that repeat with fixed time periodicity; and the irregular component, consisting on the residuals of the time series after the allocation into the seasonal and trend component (Metcalfe and Cowpertwait, 2009; Lafare et al., 2016). After this, an Autocorrelation test was performed to con-

Fig. 1. Choropleth map of the distribution of animal rabies in Metropolitana region, Chile, between the years 2003 and 2013. The intensity of colors is proportional to the number of cases from light gray to dark gray.
firm seasonality, using a correlogram, for the visual examination of correlation between monthly measures, in this case monthly frequency of rabies cases (Dolhoo et al., 2009) and the correlation values were then tested with a Ljung-Box test to confirm that there is statistically significant evidence for the presence of non-zero correlation at the defined period, confirming seasonality (Ljung and Box, 1978). All data storage, spatial analysis and mapping was performed using Microsoft Excel® 15.12.3, QGIS 2.8.2 Wien (QGIS-Development-Team, 2012), GeoDa 1.6.7 (Anselin et al., 2006) and R version 3.3.1 (R-Core-Team, 2016).

3. Results

Between 2003 and 2013 (inclusive), 927 rabies cases were reported in Chile, only two of them in domestic animals, with an annual mean and median of 84 and 86 cases per year, respectively. The vast majority of cases (98.5%) were obtained from passive surveillance rather than active surveillance, of which around 7% of the samples from passive surveillance were positive. Only 1.5% of samples collected by active surveillance were positive (Table 1). Most cases (88.1%, 816 cases) were detected in the central zone; with 2.8% (26 cases) in the north zone, and 9.1% (85 cases) in the south zone. The regions with highest numbers of reported cases were: 1) Metropolitana (376 cases, 40.6% of the total), where the communes of Las Condes (77 cases), Santiago (44 cases), Providencia (31 cases), Maipú (21 cases) and Puente Alto (21 cases) showed the highest number of animal rabies cases; 2) Valparaíso (177 cases, or 19.1% of the total) where the communes of Viña del Mar (33 cases), Valparaíso (24 cases), San Felipe (21 cases), Quilpué (18 cases) and Quillota (14 cases) showed the highest number of animal rabies cases; and, 3) Maule (109 cases, or 11.8% of the total) where the communes of Talca (36 cases), Linares (25 cases), Curicó (15 cases), Rauco (6 cases) and San Clemente (4 cases) showed the highest number of animal rabies cases – all three of which, as would be expected, are within the central zone. Choropleth maps were constructed using this information in order to have a geographical reference of the commune distribution, observing a level of clustering or neighbourhood effect in the number of cases by commune (Figs. 1–3).

Frequencies of viral detection from different host species are detailed in Table 2, observing that the main reservoir of animal rabies cases corresponds to bats, particularly *T. brasiliensis* (92% of all cases). Table 3 demonstrates the frequency of infection of bats with different viral strains, showing that viral variant 4 was the most common variant identified between 2008, year in which ISP began to register the viral variant, and 2013. The LISA test gave a Moran’s I index of 0.1537 for the Central zone, with 95% confidence intervals equals to 0.1141–0.1933, showing a *p*-value of 0.02. No significant SA was seen in North and South zones (Table 4).

Within the Central zone, LISA test for the three regions with the highest numbers of detected infected individuals (Metropolitana, Valparaíso and Maule) gave a Moran’s I index of 0.0857 and 0.0977 for Metropolitana and Valparaíso region respectively, while Maule region had a negative Moran’s I index (−0.0868). However, none of these results showed statistical significance, keeping the commune as the unit of interest.

The number of rabies detections generally decreased between May and August (late Fall and Winter), with an average of three
diagnosed cases in comparison with up to 13 cases between December and March (late Spring and Summer) (Fig. 4). The time series factor decomposition can be observed in Fig. 5. The highest detected seasonal factor is for March (about 6.79), and the lowest is for July (about −5.65), indicating that peaks in the report of rabies cases on bats occurs during hot season (October–March) and a marked decrease of reports in cold season (April–September) each year. Autocorrelation analysis shows correlation between months belonging to the same season (positive autocorrelation) and with the months of the year that belong to the opposite season (negative autocorrelation) (Fig. 6). This supported by the Ljung-Box test indicating that autocorrelation values from this time series are statistically significant and truly non-zero values ($X^2 = 234.85$ and $p$-value < 0.0001).

4. Discussion

The current study is an analysis of rabies surveillance data collected throughout Chile between 2003 and 2013. As is clear from Table 2, the vast majority of detections are from species of bats, with only two detected cases from domestic animals (one dog, one cat). This suggests that the rabies virus remains endemic in bats, as was described in 1985 (Favi et al., 2011), and suggests that bats are the main reservoir of rabies virus in Chile. These results are comparable to studies conducted between 1989 and 2005, which reported 719 cases in bats, 15 in domestic species and only one case in humans (Favi et al., 2008). The rabies virus was detected in five of the eleven species of bat known to occur in Chile, all of which are insec-

tivorous: *T. brasiliensis, L. cinereus, H. macrotus, L. borealis* and *M. chiloensis*, with most detections from *T. brasiliensis*. Antigenic characterization studies performed between the years 1977–2008 also indicated *T. brasiliensis* as the most important reservoir of rabies virus in Chile (Favi et al., 1999; Yung et al., 2012). This species of bat is distributed throughout the country (especially between regions of Arica and Parinacota and Los Ríos), and has a great capacity to adapt to different climatic conditions. *T. brasiliensis* is also strongly associated with urban centres, particularly to human habitations, forming colonies of thousands of individuals during the breeding season, usually located on roofs and cracks in constructions and buildings (Canals and Cattan, 2008). *L. cinereus* and *H. macrotus* have more solitary habits, and have a more restricted geographical distribution: *L. cinereus* in the regions of Coquimbo and Los Lagos, and *H. macrotus* between the regions of Arica and Parinacota and Biobío (Iriarte, 2007).

Rabies detections were most common in major urban areas within each region, in particular within the regions of Valparaiso, Metropolitana and Maule (Figs. 1–3). This likely represents the distribution of the main reservoir, *T. brasiliensis*, as described above. This finding is consistent with studies that analysed the spatiotemporal distribution of bats detected positive for the virus in Metropolitana region and concluded that cases in the region are concentrated in the eastern sector, which correspond to the most populated areas of the region (Hott, 2002). The three regions of most frequent detection also presents the highest human population density according to the last census (Iriarte, 2007), which may
result in a more efficient passive and active surveillance system than less densely populated regions, the case of rural areas.

Data on different rabies virus variants were obtained from the ISP antigenic typing performed by a panel of eight monoclonal antibodies, and are only available from 2008. In Chile ISP have identified 4 antigenic patterns, which link a rabies case to a specific reservoir species (Table 3). Virus variants 1 and 2 are linked to canine rabies variants, while the rest of the variants are linked to bats species, in particular variant 4 is linked to T. brasiiliensis. However, in H. macrotus serological testing failed to identify the viral variant, but genetic sequencing techniques has allowed to characterize the variants circulating in Chile within this bat species (Canals and Cattan, 2008).

Investigation of the temporal trends in prevalence of different viral variants over time supports the hypothesis that the classical cycle of rabies, which involves domestic dogs and is caused by canine variant 1, has been interrupted since 1990 (MINSAL, 2010), reaffirming the self-statement of a country free of human rabies transmitted by dogs declared to the Pan American Health Organization (PAHO), the World Health Organization (WHO) and the World Organisation for Animal Health (OIE). Although positive detections were made in two domestic animals, these corresponded to antigenic variants derived from insectivoros bats and are not thought to be associated with each other in any other way (despite appearing in the same commune Curicó at around the same time) (MINSAL, 2007).

An investigation of possible spatial autocorrelation was undertaken in the current study. Because some reports were made retrospectively, they could not be precisely geo-referenced, and so all data were aggregated by commune for this analysis (which was based on the Moran's I index). This approach assumes that the number of virus detections would be homogenously distributed throughout each commune, loosing the effect of neighbourhood or actual spatial distribution (Pfeiffer et al., 2008). There was only evidence of spatial autocorrelation in the Central zone, and this was not identified in the North and South zones, probably due to the low numbers of detections in this two zones (Bronner et al., 2015). Further inspection for spatial autocorrelation within the Central macro-zone, particularly on the three regions with most detections within this zone, did not find any evidence of spatial autocorrelation. A potential explanation is related to the effect of a zero-inflated spatial count data set that, due to low number of reported cases in this regions, affecting the statistical analysis (Agarwal et al., 2002).

Spatial aggregation of rabies has been detected in other countries: Brito-Hoyos et al. (2013) showed the existence of cluster or clusters of cases of rabies in cattle in Colombia, transmitted by bats in certain municipalities while much of the rest of the territory was free of outbreaks. Guo et al. (2013) studied the situation of rabies in China, reporting clusters of cases of human rabies, specifically in Southeast China related to canine variants, registering not only spatial but also temporal cluster and persistence of outbreaks in the affected regions.

Escobar et al. (2015) evaluated the spatial patterns of rabies virus in Chile, detecting clustering of rabies cases in large urban centres from regions with a high number of reports. Additionally, studies carried out between 1989 and 2005, determined that the largest number of cases of animal rabies were reported in the regions towards the centre of the country, namely Metropolitan, Biobío and Valparaiso regions (Favi et al., 2008). Research involving animal rabies cases from the Metropolitan region between 2000 and 2002 established the presence of clusters in main urban centres (Hott, 2002).

Descriptive temporal analysis of rabies virus detections showed an increase in diagnosed cases between October and March and a
However, the program, and their contact with infected bats, resulted in outbreaks of rabies. The importance of considering seasonal variation amongst the animal hosts of the rabies virus (Iriarte, 2007; Favi et al., 2011) is evident in the increase in the number of cases on the last years of the study; the estimated seasonal component (third from top), showing a clear peak in the hot season (September to March) and an important decrease in the cold season (April to August); and the random component (bottom), the residual after allocation into the seasonal and the trend component of the time series.

The vast majority of positive virus detections (99.2%) in the current study resulted from passive surveillance (based upon reports by individuals), which demonstrates the value of this data stream in the detection of new cases (Ouagal et al., 2010; Schatz et al., 2014). However, it is important to continue with an active surveillance program, such as the National Program for Prevention and Control of Rabies, which allowed to decrease substantially the number cases of both human and animal rabies over time, since its establishment in the 1960s, when rabies was endemic in Chile. Such programs allow the application of a risk based approach and supports the prevention of new human cases, detecting high risk zones and outbreaks and correct use of immunization (Picard-Meyer et al., 2011; Knobel et al., 2013; Ferguson et al., 2015). Is important to keep the condition of country free of human rabies transmitted by dogs, involving canine variants of the virus, especially considering the high density of dogs in Chile, approaching three million, 75% of which are free roaming (Ibarra et al., 2003). Considering also that this species has a great ability to travel long distances and have particular social behaviour (Scortti et al., 1997; Hampson et al., 2008; Acosta-Janeit et al., 2010), there is the potential risk of introduction of canine rabies from neighbouring non free-countries such as Bolivia, Argentina and Peru (PAHO/WHO, 2016), causing a re-emergence of rabies in dogs and thus, increasing the risk of human rabies cases from dog bites (Morters et al., 2014; Astorga et al., 2015). An interesting approach could be the output-based standards for surveillance, which indicate what surveillance must achieve (e.g. level of confidence, design prevalence), allowing the systems to be comparable and harmonised, adapting surveillance to different populations reality (Cameron, 2012). The case of Chile, this may allow the use of risk-based sampling, directed to those areas where bat-borne rabies is detected on a regular basis or where it could have a greater impact in Public Health.

5. Conclusions

From the data analysed it is possible to conclude that the epidemiological situation of rabies virus in Chile remains largely unchanged in recent years, being primarily endemic in insectivorous bats, with sporadic and isolated cases in domestic species associated with bat variants.
No evidence of circulation of the canine virus variants (variants 1 and 2) was detected during this study, and the largest reservoir species still appears to be the bat T. brasiliensis.

Spatial clusters of virus detections were observed in the Central region of Chile, which appeared to be associated with large urban centres. Some evidence of seasonality in the spring-summer period was detected, but further analysis is needed to characterize this. Most of these spatial and temporal aspects of virus detection are thought to be associated with the behaviour of the major reservoir host, T. brasiliensis.

The vast majority of viral detections resulted from passive surveillance, which although useful, can be challenging to interpret. However, it is also vital to maintain a system of active surveillance of rabies in Chile, as the National Program for Prevention and Control of Rabies, sampling different populations of different bat species and free-roaming dogs, this because, active surveillance is directed to increase the probability of detection of the agent and thus allowing an early detection and response, decreasing the risk of human infection. Despite the low frequency of cases in other species caused by bats variants, including human cases, the risk remains high due to the significant impact of the occurrence of rabies. The surveillance program provides valuable information on the epidemiological behaviour of the virus which supports prevention and control activities.

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