The Application of Spatial Analysis Tools in Small-Ruminant Brucellosis Eradication Programs in Northern Spain

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ABSTRACT

Information routinely collected from Official Eradication Campaigns against small-ruminant brucellosis (SRB) in a northern province of Spain was used to evaluate the usefulness of spatial analysis techniques for implementing new eradication strategies. Clustering of SRB between 1997 and 1999 was investigated by combining two techniques, namely, the population-adjusted Oden's Ipop and the spatial scan statistic. Both methods showed significant spatial clustering of cases in each of the three years. The location of significant clusters detected by scan statistic mostly matched with those areas with higher seroprevalence. Although the sensitivity of this analysis was relatively high, caution is advised regarding its specificity. A careful study of each of the clusters identified is recommended before uniformly implementing new activities within the identified risk zone. Some areas were consistently identified as clusters over the three years, suggesting that spatial analyses may allow for the comparison of disease clusters over years.

INTRODUCTION

Small-ruminant brucellosis (SRB) caused by *Brucella melitensis* is present in most of the Spanish regions.[1] Control of this infection was initiated in 1976 through mandatory vaccination (Rev-1 strain) of all replacement animals less than 6 months old.[2] In 1990, the control program was implemented with a test-and-slaughter policy.[3] Since then, the incidence of disease has significantly declined. Small-ruminant brucellosis is currently present at low individual levels, but is widespread across the country with final eradication yet to be accomplished. Thus, the removal of the mandatory vaccination program, the final step before reaching the freedom-from-disease status,[4] is still not advisable.[5]

Figure 1. Geographic location of the province of León, NW Spain.

The ongoing cost of SRB infection and control in Spain is significant. The Spanish small-ruminant census, more than 26 million heads, accounted for almost 25% of the European Union (EU-15) census in 1999.[6] The cost of SRB in Spain, therefore, comprised a significant piece of the EU animal health budget.[7] Moreover, a total of 1,548 cases of human brucellosis (HB), most of them due to *B. melitensis*, were diagnosed in 1999 in Spain.[8] The decrease of HB incidence during the last decade (from 4,217 cases in 1989) has paralleled the decline of animal brucellosis, thus suggesting the need for the eradication of animal brucellosis if human brucellosis is to be eradicated.

The standard brucellosis control programs based on vaccination of replacement animals followed by the testing of adult animals and the slaughter of the seropositive have had limited success.[9] Several problems may be associated with the failure of these programs, including the use of imperfect diagnostic tests[10,11] and the absence of both proper knowledge of the organizational structure of the livestock industry and suitable legislation.[12,13] Therefore, the understanding of the epidemiology of this endemic low-prevalence infection in Spain must continue.[5] Different studies have looked at factors that may be associated with the presence of SRB in different areas of Spain.[14-17] The identification of flocks with these factors has been considered important to halting the spread of this infection in those areas.

Endemic diseases such as brucellosis are characterized by occurrences that are usually unlimited in time but limited in space.[18] Spanish brucellosis eradication programs collect basic information at both the individual and flock levels that do not account for the temporal distribution of the disease but allow for the location of seropositive animals. Thus, in this study, we use data from the official eradication program (OEP) to investigate the usefulness of spatial analysis techniques for implementing new eradication strategies in the province of León (NW Spain). The geographic distribution of SRB and HB were also compared.

MATERIAL AND METHODS

Data Source

The data for this study were obtained from the OEP carried out in the province of León, NW Spain, during 1997, 1998, and 1999 (Figure 1). This province comprises an area of approximately 15,500 squared kilometres, and in 1998 it had a small-ruminant census of 797,514 sheep (2,902 flocks) and 53,126 goats (364 flocks).[19]

Basic data for this study, namely, population sampled, number of seropositive animals, animal species, type of production, and flock size (the number of animals >18 months of age in each flock) were retrieved from individual-flock sheets kept by the regional authorities (Consejería de Agricultura y Ganadería de la Junta de Castilla y León). Every year the campaign runs from March to June/July. All flocks for which the OEP-flock sheet between 1997 and 1999 was available were included in this study. More detailed information about this province and its OEP is presented elsewhere.[16,17]

Geographic information on all cases of HB that occurred in the province between 1998 and 1999 were also available from the regional Epidemiology Service (Consejería de Sanidad, Comunidad de Castilla y León).

Analysis of Spatial Clustering

Because the exact location of each small-ruminant flock was not available from

the OEP, flocks were georeferenced to the municipality in which they were located. The longitude and latitude of the municipality centre was used as the centroid.

First, a global cluster detection method (the population-adjusted Oden's Ipop technique) was used to test whether or not clustering of SRB existed. Oden's Ipop technique is a modification of the Moran's I method that adjusts for different population densities in each municipality.[19] It evaluates whether a spatial pattern exists in the data, regardless of location, that is unlikely to have arisen by chance. The procedure was performed using a commercial software program (Clusterseer®, TerraSeer, Inc., Ann Arbor, MI). The significance of the Oden's Ipop statistics is assessed using either a *z*-score or Monte Carlo randomization, the latter being used when data may not be normally distributed.

The spatial scan statistic[21] implemented in SatScan®,[22] was further used to identify and locate significant spatial clusters of SRB in the province of León. Briefly, this method draws a circular window centred on each of the centroids with its radius varying continuously from zero to an upper limit that was set as less than 50% of the total area. The program tests the hypothesis that animals within a particular window have the same risk of being seropositive as animals outside the window, and the primary cluster is that with the largest likelihood ratio. Secondary clusters, or clusters with smaller likelihood ratios, are also identified.

A Poisson-based model was used for this analysis. Cases in each location (municipality) are assumed to have a Poisson distribution. The population at-risk for each centroid is determined as the sum of all the animals tested during the OEP in each municipality. The model assumes that, under the null hypothesis, the expected number of cases in each area is proportional to its population size.[21] The observed number of cases in each municipality is then compared to its expected number of cases and a relative risk is estimated. The Poisson model also allows for the investigation of disease clustering while controlling for potential confounding by indirect standardisation.[22] In this study, data were analyzed adjusting for flock size (categorized according to quartiles as ≤125 head, >125 and ≤215, >215 and ≤334, and >334), type of production (milk or meat), predominant animal species (sheep or goat), and presence of other small-ruminant species in the flock (yes/no). These four variables had previously been identified as factors associated with SRB in univariable analyses.[16]

Results from the cluster analysis were further compared to choroplethic maps showing the distribution of brucellosis seroprevalence by municipality. To make choroplethic maps comparable over the three years, seroprevalence was categorized according to the 25, 50, and 75 percentiles in 1997 (≤0.06%, >0.06% and ≤0.4%, >0.4% and ≤1.6%, and >1.6%).

After identifying clusters of SRB, HB cases for 1998 and 1999 were located on the map to check for their proximity to the defined clusters.

RESULTS

More than 500,000 animals were bled in each campaign (1997, 1998, and 1999). Flocks were located in 210 municipalities within the province. These municipalities are grouped into 10 agricultural regions or *comarcas*. The number of municipalities as well as the demography of small ruminants and the seroprevalence of brucellosis in 1997, 1998, and 1999 for each comarca is shown in Table 1. Small-ruminant population was not homogeneously distributed over the province, but its distribution remained somewhat constant during these three years in most of the comarcas. A slight increase of the total population was observed over the three-year period (Table 1).

In general, SRB mean seroprevalence decreased from 1997 to 1999 (1.23%, 0.85%, and 0.63%, respectively; Table 1). Two comarcas (Tierras de León and Esla-Campos) showed higher seroprevalences

Table 1. Small-Ruminant Census and Density and Incidence of Brucellosis in the 10 Agricultural Regions Defined in the Province of León, NW Spain (1997–1999)

Agricultural Region	No. of Municipalities	Total Area (km^2)	Year	Small Ruminant Census	Small Ruminant Density	No. of Sero+ Animals	Seroprevalence (95% CI)
Astorga	20	1,393.3	1997	69,588	49.94	1061	1.52 (1.43–1.61)
			1998	69,375	49.79	882	1.27 (1.24–1.3)
			1999	71,303	51.18	355	0.50 (0.44–0.55)
Bierzo	36	2,818.8	1997	37,518	13.31	539	1.44 (1.32–1.56)
			1998	36,254	12.86	304	0.84 (0.75–0.93)
			1999	34,531	12.25	105	0.30 (0.24–0.36)
El Páramo	20	904.6	1997	65,263	72.15	826	1.27 (1.18–1.35)
			1998	67100	74.18	568	0.85 (0.78–0.92)
			1999	71,044	78.54	429	0.60 (0.54–0.66)
Esla-Campos	38	1,385.6	1997	106,460	76.83	1245	1.17 (1.10–1.23)
			1998	106,005	76.50	700	0.66 (0.61–0.71)
			1999	108,953	78.63	1,018	0.93 (0.87–0.99)
La Bañeza	17	644,5	1997	34,192	53.05	410	1.20 (1.08–1.31)
			1998	37,765	58.59	74	0.20 (0.15–0.24)
			1999	39,766	61.70	141	0.35 (0.29–0.41)
La Cabrera	7	1,277.2	1997	21,966	17.20	67	0.31 (0.24–0.38)
			1998	23,089	18.08	95	0.41 (0.33–0.49)
			1999	22,574	17.67	8	0.04 (0.01–0.07)
Montaña de Luna	13	1,963.1	1997	30,108	15.34	465	1.54 (1.40–1.68)
			1998	24,410	12.43	261	1.07 (0.94–1.20)
			1999	24,283	12.37	66	0.27 (0.20–0.33)
Montaña de Riaño	23	2,396.8	1997	34,974	14.59	259	0.74 (0.65–0.83)
			1998	37,716	15.74	294	0.78 (0.69–0.87)
			1999	35,054	14.63	63	0.18 (0.13–0.22)
Sahagún	15	923.5	1997	38,189	41.35	469	1.23 (1.12–1.34)
			1998	40,168	43.50	233	0.58 (0.50–0.65)
			1999	40,912	44.30	120	0.29 (0.24–0.34)
Tierras de León	21	1,761.1	1997	75,940	43.12	970	1.28 (1.20–1.36)
			1998	75,446	42.84	1,000	1.33 (1.25–1.41)
			1999	73,859	41.94	962	1.30 (1.22–1.38)
TOTAL	210	15,468.5	1997	514,198	33.24	6,311	1.23 (1.20–1.26)
			1998	518,328	33.51	4,411	0.85 (0.82–0.87)
			1999	522,279	33.76	3,267	0.63 (0.60–0.65)

Table 2. Results of the Oden's Ipop Analysis for Spatial Clustering of Small-Ruminant Brucellosis Cases in the Province of León, NW Spain (1997–1999)

Year	No of cases	z-Score	P-Value	Within %*	Among %*
1997	6,311	880.0	<0.001	78.0	22.0
1998	4,411	579.1	<0.001	85.6	14.4
1999	3,267	501.5	<0.001	74.8	25.2

*Percentage of estimated spatial clustering attributed to cases in the same municipality (within) and in adjacent municipality (among).

during the last year (1.3% and 0.93%, respectively) than the provincial average (0.63%). The distribution of seropositivity did not follow that of the small-ruminant population or density. For example, neighbouring comarcas with similar animal density showed different seroprevalences (i.e., Astorga or Sahagún vs Tierras de León; El Páramo vs Esla-Campos).

The Oden's Ipop method showed significant spatial clustering of SRB cases in the province for each of the three years under study ($P < 0.001$). For the three years, clustering was mostly due to the number of cases within municipalities than from cases in adjacent municipalities (Table 2). In 1997, larger SRB seroprevalences were found in central areas of the province, as well as peripheral municipalities in the west, south, and east (Figure 2). This distribution remained somewhat similar during 1998 (although with a lower number of municipalities involved), but it changed dramatically in 1999. During that year, the highest seroprevalences were observed mostly in municipalities within the central area of the province, with zero or very low seroprevalences in the rest of the province, with the exception of some isolated municipalities (Figure 2).

Spatial scan statistics also identified clusters in each of these years (Figure 2 and Table 3). In 1997, when the seroprevalence was higher, SatScan defined a large primary cluster, localized 31.2 km around the municipality of Valverde de la Virgen (42˚56" N, 5˚68" W), and 5 other secondary significant clusters. Seventy-two percent (38 of 53) of the municipalities presenting seroprevalences within the highest quartile (>1.6%) were within these clusters, and 76% of those falling within the two lowest quartiles (≤0.4%) were outside of any identified cluster (Figure 2).

In 1998, despite seroprevalence distribution that was somewhat similar to that in 1997, a different cluster pattern was observed. Twelve small, significant clusters were identified (Table 3, Figure 2). Overall, 18 (72%) of the municipalities with a seroprevalence >1.6% were included in these clusters and 96% of those with seroprevalence 0 .4% were excluded from clusters (Figure 2).

In 1999, seroprevalence was significantly lower than in previous years. A primary cluster was defined 21.8 km around the municipality of Valdefresno (42˚59" N, 5˚49" W), which was 19 km away from the centre of the primary cluster in 1997. Another four smaller secondary clusters were also identified (Table 3). One of them was comprised of two municipalities that were already included within clusters of the previous two years. Two others comprised municipalities that were within clusters in 1997. Fifteen (83%) of the municipalities with a seroprevalence >1.6% were included in the clusters detected that year, and 152 (93%) of municipalities with seroprevalence 0.4% were not within clusters (Figure 2).

A total of 68 human cases of brucellosis were diagnosed between 1998 and 1999 in this province (31 and 37, respectively). Cases were distributed over the entire province. However, it should be noted that most of them were located within or adjacent to the defined SRB clusters (55% in 1998 and 68% in 1999).

DISCUSSION

Spanish SRB eradication campaign offered a unique opportunity for the study of the epidemiology of this infection. First,

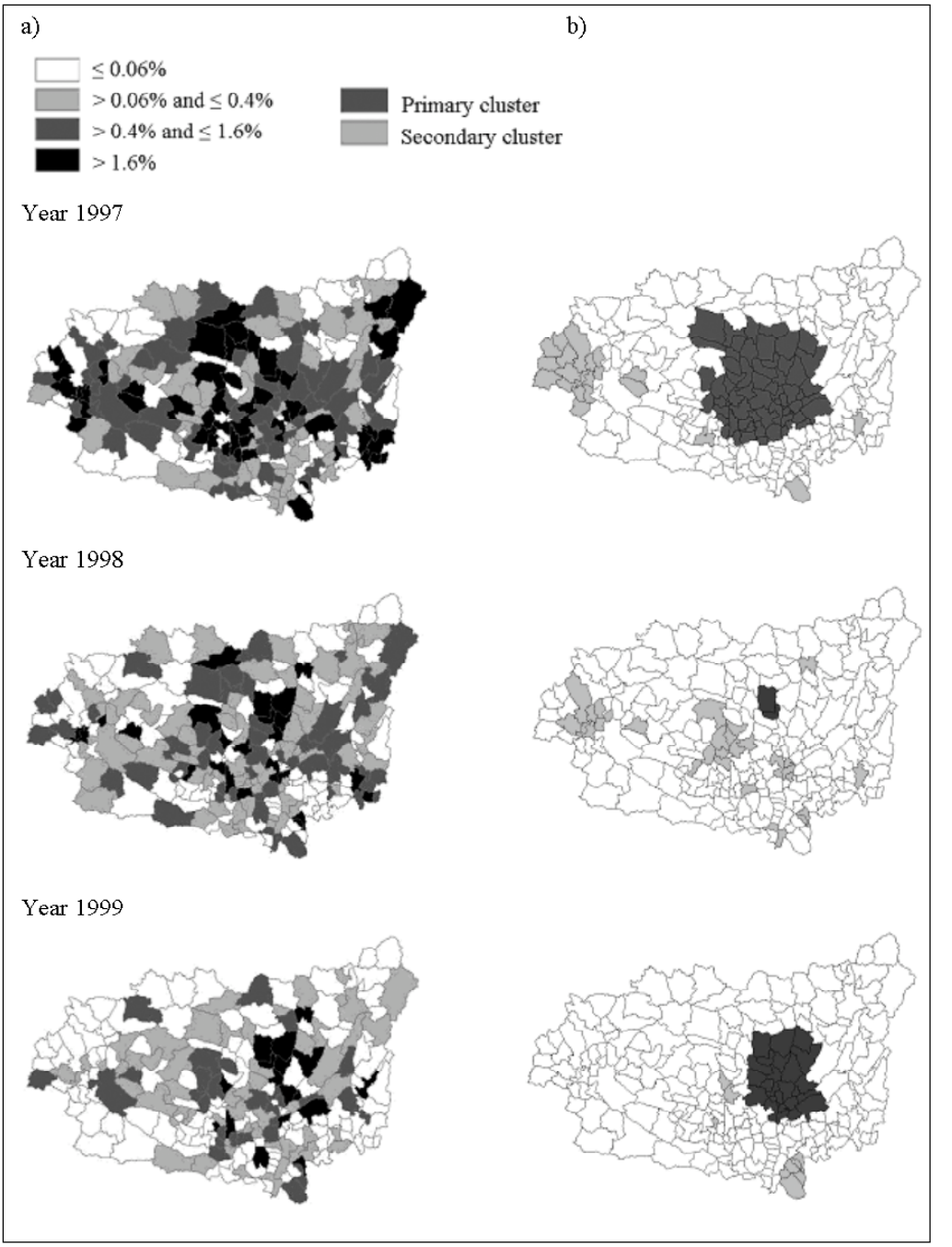

Figure 2. Distribution of small-ruminant brucellosis seroprevalence (a*) and location of significant spatial clusters (b) in the province of León, NW Spain (1997–1999). *Categories based on the 25%, 50%, and 75% percentiles for the distribution of small-ruminant brucellosis in 1997.

campaigns were census-based, so information on the population-at-risk was readily available. Second, because it was mandatory to cull infected animals from the flocks every year, timely information about the incidence of the infection was obtained as new cases of disease were detected. In addition, basic information regarding both the

Table 3. Clusters of Cases of Small-Ruminant Brucellosis After Adjusting for Potential Confounders (Flock Size, Type of Production, and Animal Species) in the Province of León, NW Spain (1997–1999)

Year	Cluster	Location	Radius (km)	Observed cases	Expected cases	RR*	P-Value
1997	Primary cluster	42˚56" N, 5˚68" W	31.2	3,272	2,185	1.5	<0.01
	Secondary clusters	42˚38" N, 5˚07" W	0	195	20	9.5	<0.01
		42˚07" N, 5˚44" W	0	300	105	2.8	<0.01
		42˚61" N, 6˚96" W	25.7	220	84	2.6	<0.01
		42˚53" N, 6˚51" W	6.2	113	28	4	<0.01
		42˚30" N, 5˚99" W	0	70	38	1.8	<0.01
1998	Primary cluster	42˚71" N, 5˚63" W	7.8	498	55	9	<0.01
	Secondary clusters	42˚87" N, 5˚40" W	0	196	15	12.8	<0.01
		42˚21" N, 5˚42" W	2.7	138	12	11.3	<0.01
		42˚50" N, 5˚89" W	11	570	270	2.1	<0.01
		42˚33" N, 5˚75" W	0	140	25	5.6	<0.01
		42˚40" N, 5˚54" W	4.3	177	41	4.3	<0.01
		42˚66" N, 6˚05" W	0	207	59	3.5	<0.01
		42˚59" N, 6˚72" W	7.9	187	54	3.4	<0.01
		42˚38" N, 5˚07" W	0	80	14	5.9	<0.01
		42˚58" N, 6˚46" W	0	61	20	3.1	<0.01
		42˚38" N, 6˚10" W	0	72	28	2.6	<0.01
		42˚14" N, 5˚59" W	0	92	50	1.8	<0.01
1999	Primary cluster	42˚59" N, 5˚49" W	21.8	1605	415	3.9	<0.01
	Secondary clusters	42˚51" N, 5˚86" W	3	316	59	5.3	<0.01
		42˚13" N, 5˚39" W	9.7	217	79	2.8	<0.01
		42˚35" N, 5˚92" W	0	45	13	3.5	<0.01
		42˚36" N, 5˚85" W	0	44	18	2.5	<0.01

*Relative risk (RR): ratio of the number of cases observed in the identified cluster and the number of cases expected in that cluster, assuming cases are Poisson distributed.

individual animal and the flock was collected and stored. Information on the temporal component of SRB infection was, however, missing as animals were sampled at times (usually once per year) that were not related to the time of infection but to the campaign schedule. Therefore, it was not possible to define temporal clusters of SRB from this type of data. The information available was used to assess its usefulness for the detection of spatial clusters only.

The scan statistic has been described as an analytical technique suitable for the identification and location of spatial clusters of diseases.[22] The latter feature is particularly appealing in the context of eradication campaigns. The proper identification of clusters of disease may lead to the implementation of new programs based on targeting the brucellosis high-risk areas. In addition, the scan statistic is especially suitable when dealing with populations of heterogeneous distribution, as happens to be the case with extensively or semi-extensively managed livestock.[23] Thus, OEP data fit very well for use with this technique.

Despite these advantages, it is recommended that other techniques of global clus-

ter detection be used in addition to the scan statistic. Global cluster detection methods (i.e., the Oden's Ipop method) have a different goal but one that complements the scan statistic when trying to detect and characterize clustering of disease in epidemiological studies.[22,23] In this study, both techniques detected disease clustering. The Oden's Ipop method showed significant spatial clustering of SRB cases mostly due to the number of cases within municipalities. This was an expected outcome since animals are grouped in flocks and this disease it is transmitted by direct contact with infected animals or their contaminated products.[24]

The location of the significant clusters detected by scan statistic generally matched those areas with higher seroprevalence, that is, those within the highest quartile (Figure 2). More than 70% of the municipalities with seroprevalences >1.6% were included within significant clusters in 1997 and 1998. In 1999, this percentage reached up to 83%. The detection of areas with high levels of infection should be a priority in OEPs since they may act as foci for new infections in the surrounding areas. This technique seemed to work quite well for that purpose.

However, caution is advised regarding its specificity. It has been previously noticed that the scan statistic tends to detect one large cluster that usually encompasses smaller clusters and also includes those areas outside the primary focus of infection that do not have elevated risk.[25] We observed this situation in both 1997 and 1999, when a large primary cluster was defined that comprised a high number of municipalities (32% and 46% respectively) with very low or zero seroprevalence (≤0.4%). Thus, a careful study of each of the clusters identified is recommended before uniformly implementing new activities within the identified risk zone. Overall results suggested, however, that as seroprevalence declined in the province, greater cluster accuracy was obtained.

The results shown in this study were obtained after adjusting for 4 potential confounders (flock size, type of production, predominant animal species in the flock, and presence of other small-ruminant species in the flock) that had been previously identified as factors associated with SRB.[16] The purpose of this approach was to prevent the identification of clusters due to a heterogeneous distribution of these four variables. Crude analyses were also run without adjusting for any of these confounders (results not shown). In the crude analyses, a large primary cluster was identified for each of the three years. Overall, similar sensitivities were obtained as compared to the adjusted analyses, but lower specificities were found. If spatial clustering is to be used in eradication programs, it may be useful to collect more detailed information on both individual animals and flocks to further increase the specificity of the analysis. Determining in advance major factors associated with the disease may help to better identify the most likely clusters of disease.

Human brucellosis is directly related to animal brucellosis. Infection is transmitted either through direct contact with infected animals or the consumption of their contaminated products.[26] Under the first scenario, overlapping areas for animal and human cases would be expected, while in the second this overlapping would not be necessarily anticipated (i.e., consumption of artisan-contaminated cheese in urban areas). In this study a larger number of cases of HB were within or adjacent to the SRB clusters previously defined, probably suggesting that direct contact with infected animals is a potentially important route of infection.

There is some potential for misclassification in these data. Flocks were georeferenced to the urban centres of their respective municipalities when, in fact, some of them were located kilometres away and sometimes located closer to other municipalities. In addition, small-ruminant management systems are mostly extensive or semi-extensive in that province,[27] with animals grazing areas belonging to different

municipalities. This would help to understand some of the discrepancies observed between SRB clusters and cases of HB. More precise geographic information on the animal premises may help to increase the accuracy of this analysis. Results suggest that the proportion of HB cases within or neighbouring a SRB cluster increased (from 55% in 1998 to 68% in 1999) as animal seroprevalence decreased; however, no cases of HB were available for 1997 to explore this hypothesis.

Overall, the spatial scan statistic can be a useful tool to use in SRB eradication programs. This technique compares the incidence of disease in a certain area to what is expected (i.e., the endemic level) in the surrounding area, thus enhancing the detection of spatial epidemics. Although the simple visual examination of incidence/prevalence maps may give a detailed distribution of the disease in a geographic area, the scan statistic allows for an unbiased detection of areas with high seroprevalence as it is not influenced by arbitrary seroprevalence categories that are, for example, an inherent problem in designing choroplethic maps based on percentiles.

In this study, some areas of the province were identified as clusters in all three years. Where resources are limited, control activities could be targeted at areas with significant long-lasting clusters of disease. Where clusters moved from one area to a neighbouring area, the reasons for that shift also could be investigated further. Spatial analyses provide useful information when used together with the human infection data to better understand the epidemiology of this zoonosis.

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