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Using Multinomial and Space-Time Permutation Models to Understand the Epidemiology of Infectious Bronchitis in California Between 2008 and 2012

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SUMMARY. Although infectious bronchitis virus (IBV) has been described as one of the most economically important viral respiratory diseases in poultry, there are few analyses of outbreaks that use spatial statistics. In order to better understand how the different genotypes of IBV behave spatially and temporally, we used geographic information system-based mapping coupled with spatial and spatial-temporal statistics to identify statistically significant clustering of multiple strains of infectious bronchitis (IB) between 2008 and 2012 in California. Specifically, space-time permutation and multinomial models were used to identify spatial and spatial-temporal clusters of various genotypes of IBV. Using time permutations (i.e., windows) spanning days to years, we identified three statistically significant ($P < 0.05$) clusters. In contrast, multinomial models identified two statistically significant spatial-temporal clusters and one statistically significant spatial cluster. When comparing the space-time permutation and multinomial models against each other, we identified spatial and temporal overlap in two of the three statistically significant clusters. From a practical perspective, multinomial clustering approaches may be advantageous for studying IB because the model allows the different genotypes of IB to be independent nominal variables, thereby allowing for a more detailed spatial analysis. To that point, based on their risk ratios, the genotypes classified as vaccine-related were identified as the most significant contributor to two of the three mutinomial clusters. Additionally, statistically significant clusters were mapped and layered on a hot-spot analysis of commercial poultry farm density in order to qualitatively assess the relationship between farm density and clusters of IBV. Results showed that one of the three space-time permutations and one of the three multinomial clusters were spatially centered near the highest density farm areas, as determined by the hot-spot analysis.

RESUMEN. Uso de modelos multinomiales y permutaciones espacio-temporales para comprender la epidemiología de la bronquitis infecciosa en California entre los años 2008 y 2012.

Aunque el virus de la bronquitis infecciosa (IBV) se ha descrito como una de las enfermedades virales respiratorias de mayor importancia económica en la avicultura, existen pocos análisis de brotes que utilicen estadísticas espaciales. Para comprender mejor como los diferentes genotipos del virus de la bronquitis se comportan espacial y temporalmente, se utilizó un mapeo basado en el sistema de información geográfica junto con estadísticas espaciales y espacio-temporales para identificar la agrupación estadísticamente significativa de múltiples cepas del virus de la bronquitis infecciosa (IBV) entre los años 2008 y 2012 en California. Específicamente, se usaron modelos de permutación espacio-tiempo y modelos multinomiales para identificar agrupamientos espaciales y espaciales-temporales de varios genotipos del virus de la bronquitis infecciosa. Usando permutaciones de tiempo (denominados ventanas) que abarcaban de días a años, se identificaron tres grupos estadísticamente significativos ($P < 0.05$). Por el contrario, los modelos multinomiales identificaron dos grupos espacio-temporales que fueron estadísticamente significativos y un grupo espacial que fue estadísticamente significativo. Al comparar entre las permutaciones espacio-temporales y los modelos multinomiales, se identificó superposición espacial y temporal en dos de los tres conglomerados estadísticamente significativos. Desde una perspectiva práctica, los enfoques de agrupamiento multinomial pueden ser ventajosos para el estudio del virus de la bronquitis porque el modelo permite que los diferentes genotipos del virus de la bronquitis sean variables nominales independientes, lo que permite un análisis espacial más detallado. Hasta ese momento, en función de sus proporciones de riesgo, los genotipos clasificados como relacionados con la vacuna se identificaron como los contribuyentes más significativos a dos de los tres grupos mutinomiales. Además, se mapearon y agruparon grupos estadísticamente significativos en un análisis de puntos calientes de la densidad en granjas avícolas comerciales con el fin de evaluar cualitativamente la relación entre la densidad de las granjas y los grupos del virus de bronquitis infecciosa. Los resultados mostraron que una de las tres permutaciones espacio-temporales y uno de los tres grupos multinomiales se centraron espacialmente cerca de las áreas agrícolas con mayor densidad, según lo determinado por el análisis de puntos calientes.

Keywords: IB outbreak, GIS, spatial statistics

Abbreviations: Ark = Arkansas; IB = infectious bronchitis; IBV = infectious bronchitis virus; Cal99 = California variant 99, CA1737 = California variant 1737; Conn = Connecticut; GIS = geographic information system; Mass = Massachusetts; NA = not available; OHS = optimized hot spot; STPM = space-time permutation model; Vacc = vaccine.

Infectious bronchitis (IB) in chickens is a highly contagious disease caused by the infectious bronchitis virus (IBV) (3). IB primarily affects the upper-respiratory tract of chickens and replicates in the epithelial tissues of the respiratory, urinary, reproductive, and intestinal tracts (2,11). Infection is associated with reduced egg production and quality in egg-type chickens and poor feed conversion and increased condemnation in meat-type chickens. One of the main limiting factors in controlling IBV is the use of nonserotype-specific vaccines that can result in the formation of novel variants (10). Specifically, these types of control efforts culminate in the generation of novel variant strains that circumvent

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vaccine-based immunity and thereby perpetuate the virus in the field (10), which results in costly and nonefficacious preventive strategies (4,20,25).

Currently, an extensive amount of research has focused on variant types (3,7,8,10,12,19,20,25), vaccinations protocols (1,12,16,17), and the relationship between new IBV variants and the emergence of disease (12,26). However, there are no studies that investigate how the different subtypes of IBV behave spatially and temporally at the population level, with respect to clustering and distribution. The use of spatial-temporal statistical models to look at infectious diseases such as IB can be an important tool in determining critical time-space boundaries with respect to IB prevention and control programs.

Among the tools available to explore questions regarding spatial-temporal disease distribution, space-time permutation models (STPMs) and multinomial models have been used for retrospective disease cluster detection of different diseases (5,14,23). Briefly, these models use "scanning windows," which move across space (spatial only), time (temporal only), and space and time (spatial-temporal) in order to identify statistically significant clusters of disease. Specifically, the scanning windows cover every possible time interval for every possible geographic location within the temporal and geographic boundaries of the study. Statistically significant clusters appear when a statistically significant number of premises are observed based on a log-likelihood ratio statistic (8). The STPM approach compares the observed cases in a cluster to what would have been expected if the cases were independent in space and time, whereas the multinomial model allows for the analysis of different case categories within a cluster (e.g., IB variants) by determining if the distribution of cases and categories in a cluster are different from the rest of the study region (13,14).

The objective of this study was to identify statistically significant clusters of IBV events in space, time, and space and time in California by using STPM and multinomial models. In addition, the distribution of commercial poultry premises (e.g., separate locations) in the central valley of California was analyzed in order to better understand the relationship between density of farms and disease events. The results of this study and the practical application of these geographic information system (GIS)-based statistical mapping models can be used to make targeted biosecurity and IB vaccine recommendations to farmers.

MATERIAL AND METHODS

IBV cases. Out of the 1444 cases of IBV identified in California via passive surveillance between 1997 and 2012, 131 IBV cases (9.1%) were identified with address information and, hence, were included in this study. Positive cases with address information were diagnosed by the four California Animal Health and Food Laboratory System labs during the period between September 1, 2008, and December 31, 2012. Diagnoses were performed from samples obtained from birds with clinical signs and/or with gross and microscopic lesions consistent with IBV. Virus identification was done by reverse transcriptase–polymerase chain reaction targeting the hypervariable region of the spike protein subunit 1. Products were submitted for sequencing, and sequences were analyzed using Mac Vector 14.0. (MacVector, Inc., Apex, NC). Sequence percent homologies were calculated after comparing them against the GenBank NCBI database (Bethesda, MD), which is able to detect variations in the spike protein subunit 1 to determine different strains and variants. Virus isolations and identification were done following the standard techniques described by the American Association of Avian Pathologists (6). IBV genotypes identified in our study included California variant 99 (Cal99), California variant 1737 (CA1737), Massachusetts (Mass), and Connecticut (Conn). To represent the effects of vaccine-related strains, Conn and Mass were grouped in a single category named "vaccines" (Vacc). Cases without information regarding IBV strains or cases that did not match sequences in GenBank were classified as not available (NA). Additional data collected that were linked to each IBV variant included date of submission, IBV genotype, and submitter.

Spatial distribution of IBV. Using address data collected and recently updated (2017) by a regulatory agency, we georeferenced all commercial poultry farms within California and performed an analysis using the Optimized Hot Spot (OHS) analysis tool in ArcGIS® version 10.2. The OHS analysis identifies statistically significant hot spot and cold spot clusters by aggregating the incident cases (i.e., poultry premises in California) into fishnet polygons that are positioned over the study region (18,22). The size of each cell in the fishnet is computed from an algorithm considering both the average and median nearest neighbor distances among premises, adjusted by the lowest and highest distances among premises. The premises within each cell were counted, the cells with zero values were removed, and the remaining cells were analyzed. The number of premises within each cell was used to calculate the Getis-ORD Gi* statistic for each polygon. This is a spatial statistic that produces a z score for each cell in order to determine the significant hot spots (i.e., high z score) and cold spots (i.e., low z scores) in the study area (9,22). Specifically, the analysis works by considering each cell feature in terms of neighboring features, meaning that a cell with high values of premises needs to be surrounded by other cells with high values to be a statistically significant hot spot. In addition, descriptive statistics such as the mean count of premises in each cell, number of hot spots, and the mean number of premises in the hot spots were also calculated.

Spatial and space-time analysis. In order to visualize the distribution of IBV cases in California during the study period, each positive case was spatially associated with the company performing the submission. All cases were mapped using this approach. To investigate statistically significant IBV clusters in space and time, a STPM was performed using SaTScan™ version 9.1.1 (National Cancer Institute, Bethesda, Maryland, USA). Using the location of the company submitting laboratory samples, we fit the models by comparing the numbers of observed cases in a given cluster with the number of expected cases, with the assumption that the spatial and temporal locations of all cases have no space-time interaction (i.e., independent in time and space). The analyses were executed considering a daily, monthly, and yearly time aggregation for cluster length. Finally, to explore the spatial and spatial-temporal clusters in a single analysis at the genotype level, a multinomial scan analysis was performed via SaTScan (National Cancer Institute). Specifically, the analysis included the genotypes Cal99 and CA1737, the vaccine-related strains Conn and Mass (i.e., Vacc), and the NA cases. The statistical significance of "most likely clusters" (i.e., clusters that are least likely due to chance) in all the models fitted were evaluated through Monte Carlo simulations (999 replications), and P values <0.05 were considered statistically significant to reject the null hypothesis of random distribution in time or space. The maximum spatial cluster size was set at 50% of the population at risk.

RESULTS

A total of 798 premises were included in the OHS analysis to describe the spatial distribution of poultry premises in California and to investigate the presence of high-density clusters (Table 1; Figs. 1, 2). The area of each polygon created in the fishnet mesh during the OHS analysis was 9.57 km^2, with a total of 240 polygons and a mean count of premises per polygon of 3.325 (SD, 4.17;

Table 1. Genotypic distribution of the 133 IBV cases used in this study in California between 2008 and 2012.

IBV genotype	*n*	%	95% CI
Cal99	63	48.1	39.3–56.9
CA1737	4	3.1	0.9–8.1
Conn	18	13.7	8.5–21.1
Mass	3	2.3	0.6–7.1
NA	43	32.8	25.02–41.6

range, 1–33). The OHS analysis was able to detect 47 statistically significant polygons ($P < 0.01$, $n = 44$; $P < 0.05$, $n = 3$) constituting one hot spot area located in the central valley of California (Figs. 1, 2). The area has a mean of 7.34 premises per polygon (SD, 7.15; range, 1–33) and an area of 56.37 km^2.

Among the 131 IBV-related cases included in the study, genotypic identification was performed on a total of 88 cases (Table 1). Ninety-seven percent of the cases represented broilers ($n = 127$, 95% CI = 91.8–99) and 3% represented layers ($n = 4$, 95% CI = 0.9–8.1).

The STPM identified six space and time clusters (Table 2), including three that were statistically significant ($P < 0.01$). All three significant spatial-temporal clusters were detected in at least two time-extent aggregations, with durations of 131, 65, and 179 days and radii of 15.78, 33.87, and 32.25 km, respectively (Table 2). The odds ratios (i.e., ratio between observed and expected cases) among the significant clusters were 6.75, 4, and 2.27, respectively. Broiler premises were predominant in all the significant clusters obtained in the STPM analysis (Table 2).

With respect to the identification of spatial-temporal clusters by using the multinomial model, results showed significant clusters for different IBV types during the study period (Table 3). Specifically, the spatial-temporal analysis showed two statistically significant clusters ($P < 0.01$). In the first cluster (i.e., cluster 1 from the spatial-temporal analysis), the category Vacc had the highest relative risk (2.68) within the different genotypes of IB (Table 3). However, Cal99 ($n = 7$) was the most predominant in the cluster with respect to the total number of cases (Table 3). In contrast, the second significant spatial-temporal cluster (i.e., cluster 2 from the spatial-temporal analysis) was conformed only by NA cases (Table 3). With respect to the spatial analysis, two clusters and one statistically significant cluster was obtained ($P < 0.01$), and it had Cal99 and Vacc (Conn and Mass) as its more predominant genotypes and had the highest relative risk.

With respect to the identification of spatial clusters by using the multinomial model, results showed two clusters with only the second cluster being statistically significant ($P < 0.05$) (Table 3). Within this cluster, the Vacc genotype had the highest relative risk (2.55) (Table 3). However, Cal99 was the most predominant in the cluster, with respect to the total number ($n = 29$) of cases (Table 3). In both analyses, broiler farms were the most represented in all the significant and nonsignificant clusters (Table 3).

DISCUSSION

Due to its economic and poultry health impact, IB is a well-studied disease in poultry (1,12,26). In contrast, the spatial epidemiology of IB is not well studied, especially in contrast to other poultry diseases including avian influenza (15). The ability to identify statistically significant spatial, temporal, and spatial-temporal clusters of infectious disease is an important tool in public health, disease surveillance, and food security (15,21). With the advent of mapping tools and space-time disease surveillance software, timely retrospective analyses of disease outbreaks can be accomplished with little or no cost. Among the commonly used space-time disease surveillance tools, which include ClusterSeer, SaTScan, Geosurveillance, and the surveillance package for R, we selected SatScan due to its open-source nature, ease of use, well-referenced methodologies section, and the variety of analyses tools available (13,14,24).

In this paper, we explored several spatial statistic approaches in order to explore practical ways to facilitate the identification of clusters of IB. Specifically, based on previous efforts in mapping infectious diseases, combined with the high variability of IB genotypes noted in California (8), STPM and multinomial models were identified as appropriate retrospective tools to properly identify spatial-temporal clustering of IB during the study period (5,23). These spatial-temporal statistic tools are helpful in identifying both geographic locations and times of year in which IB variants are most likely to occur.

Using the space-time permutation, we found statistically significant clusters under three time aggregation windows (day, week, month, year; day, week; and month, year) (Table 2). This demonstrates the utility of using the time window at multiple temporal scales.

Clusters number two and six overlapped each other in both time and space (Table 2; Fig. 1). One limitation of the time-space permutation is that there is no specificity as to the IBV genotype. Specifically, we cannot identify if Cal99 or the Vacc cluster (i.e., Conn and Mass), for example, is the primary cause of the cluster. Hence, without genotypic information, it is more difficult to understand the interrelatedness of outbreaks that overlap in space and time (i.e., cluster number 2 and 6 in Table 2; Fig. 1). Therefore, an understanding of the clustering patterns of the different IBV variants known in California (8) is not captured. This is particularly important with IB, because vaccine strains are commonly identified in poultry houses in addition to wildtype strains. Hence, we explored this by using spatial-temporal and spatial analyses via a multinomial model, which, by definition, allow for the categorization of the different IB genotypes (Fig. 2; Table 3).

Using this approach, we identified two significant spatial-temporal clusters and one significant spatial cluster (Table 3; Fig. 2). With respect to genotype, results showed that in cluster 1 on the spatial-temporal analysis and cluster 2 of the spatial analysis, the vaccine strains had the highest relative risk (Table 3), meaning that more cases of IB associated with the vaccine strain are observed than expected. Consequently, it appears that the vaccine strain has a more significant impact than what was expected. In addition, these clusters (e.g., cluster 1 from the spatial-temporal analysis and cluster 2 from the spatial analysis of Table 3) are roughly analogous in space and time to clusters 2 and 6 in Table 2 (i.e., the STPM). Because both clusters were both primarily associated with high relative risks for the vaccine-like strains, this brings up the likelihood that both models detected similar clusters.

Although there was more than one genotype in each cluster, it is important to note that significant relative risks were found only for one IB genotype (Table 3) for each cluster. Following the vaccine strain, the NA strains (e.g., cases without information or Genbank

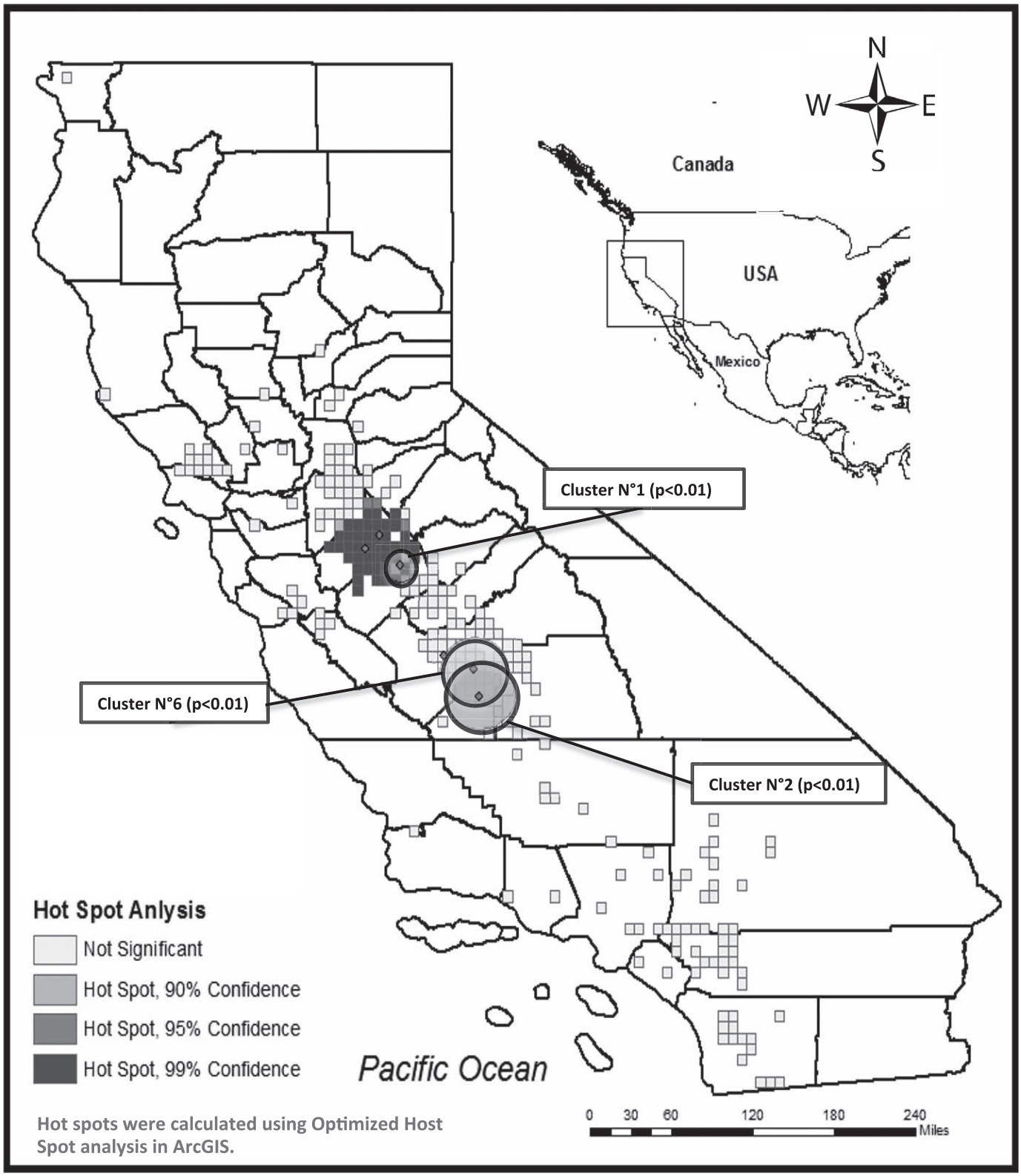

Fig. 1. Clusters of IBV in central California obtained by the STPM by using day, month, and year as time aggregation (2008–2012) and OHS analysis of poultry producers in California. Producer's locations were mapped in 9.6-km^2 grids. Additional information regarding each cluster can be found in Table 2.

matches) had the next highest relative risks. The California genotypes Cal99 and CA1737 had relative risks below 1 (i.e., they were seen less than expected) for every cluster except for cluster 2 of the spatial analysis, where it was just above 1 (1.34) (Table 3). With respect to the NA strains, they include more than one genotype that do not match GenBank sequences. Hence, further analysis of those NA genotypes would be required to evaluate the genetic variability of each genotype.

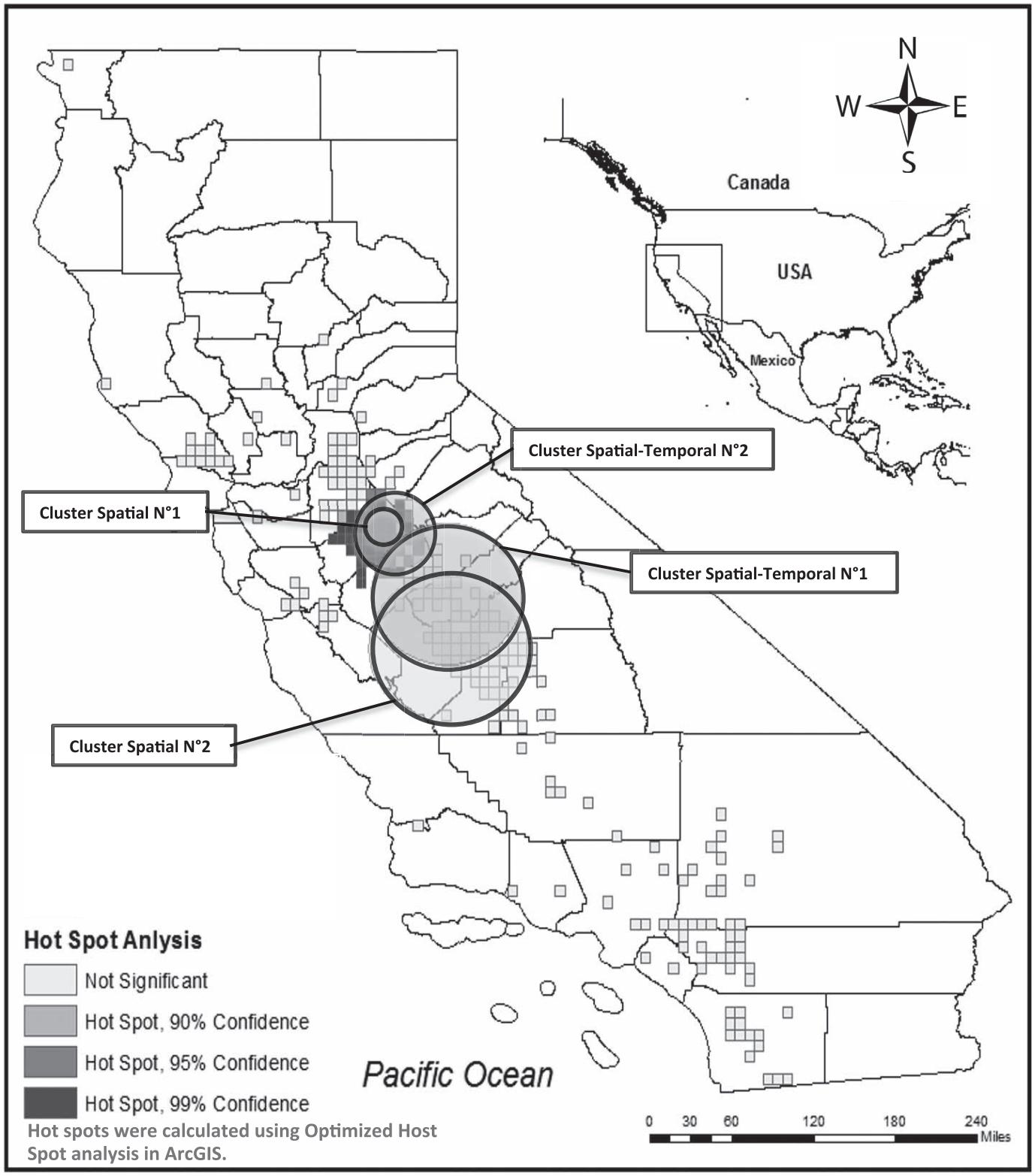

Fig. 2. Spatial-temporal and spatial clusters fitted by a multinomial model for a given variant of IBV in California (2008–2012) and OHS analysis of poultry producers in California. Producer's locations were mapped in 9.6-km^2 grids. Additional information regarding each cluster can be found Table 3.

For IB disease surveillance, the above insights regarding the vaccine-like viruses demonstrate the inherent advantage of multinomial models with respect to the identification of different genotypes of IB. Specifically, in addition to the geographic and temporal parameters of an IB outbreak, genotypic information can also be provided to help facilitate response efforts. One interesting potential disadvantage of the multinomial model in this study is that the geographic areas and time periods where clusters were identified

Table 2. Clusters of IBV in central California obtained by the STPM by using day, month, and year as units of time aggregation (2008–2012).

Cluster	Time aggregation	Radius (km)	No. of producers (broilers/layers)	Start date	End date	*P* value	Cases	Expected	Cases/Expected
1[A]	Day, week, month, year	15.78	20/1	May 10, 2011	Sep 21, 2011	0.0004	9	1.33	6.75
2[A]	Day, week	33.87	24/2	Nov 17, 2008	Jan 22, 2009	0.0031	13	3.25	4
3	Day, week, month	14.94	9/1	Sep 27, 2011	May 1, 2012	0.301	8	1.76	4.55
4	Day	0.34	4/0	Dec 19, 2008	Dec 19, 2008	0.436	2	0.039	51.11
5	Day, week, month	5.71	12/10	Jan 11, 2010	May 4, 2010	0.954	3	0.24	12.6
6[A]	Month, year	32.25	60/2	Oct 1, 2008	Apr 30, 2009	0.0031	27	11.87	2.27

[A]Statistically significant clusters.

were much larger spatially and, in one case, temporally (i.e., 4 years for spatial cluster 2 from the multinomial model in Table 3), than the corresponding geographic areas for the space-time permutation (Table 2). This could be because the multinomial model evaluates whether there are any clusters where the distribution of cases (distribution of categories, in our case, distribution of IB types) is different from the rest of the study region, whereas the STPM model compares the observed cases to what would have been expected if the spatial and temporal locations of all cases were independent of each other.

One potential approach toward future analysis of IB outbreaks could be to use the multinomial models to identify the clusters of disease and then use the space-time permutation to better understand geography and time. This would narrow down the time and space frames in addition to providing relevant genotypic data.

The secondary objective was to qualitatively understand the relationship between farm density and clusters of IBV. Of the three significant space-time permutations, only one of the clusters was geographically located in an area of high poultry farm density, as identified by hot-spot analysis (Figure 1). Likewise, with respect to the multinomial models, only one of the three statistically significant clusters (spatial-temporal cluster 2) was spatially centered near the highest density farm areas, as determined by the hot-spot analysis (Figure 2). These results most likely reflect the reality that other factors (e.g., roads, wind, and biosecurity practices) in addition to farm density contribute to clustering of disease. Further studies integrating these types of variables in addition to geography should be considered as a next level of analysis, with the goal of clarifying the root causes of the outbreak.

Unfortunately, of the 1444 IBV cases identified, only 131 (9.1%) contained address information. Hence, over 90% of the data was not analyzed spatially. This could result in some type of selection bias (e.g., companies that do or do not fill out the complete address information on the submission form). An additional limitation of the current study is that there is no specific information provided as to the vaccines that were used. In California, although commercial egg-layer premises use Mass, Conn, and Arkansas (Ark) IBV vaccines during the rearing period and sometimes during production, broiler premises may use Mass and Conn, many premises have been migrating away from field vaccination and even hatchery priming (C. Corsiglia, pers. comm.). This is interesting because, as we move away from vaccine boosters in broilers, we have noted that IBV outbreaks tend to spread in time and are usually linked with the use of Ark vaccines in egg layer-facilities (R. Gallardo, pers. comm.). The data set analyzed reflect stationary problems that might be associated with the use of certain vaccine strains (Table 3; Fig. 2). Specifically, this was reflected in two of the three significant multinomial clusters where the vaccine strains had the highest risk ratios (i.e., were found more often than expected). Because the use of certain vaccine types (i.e., Ark DPI) promotes the generation of even more variants (8), this type of epidemiologic analysis is important to help facilitate better decision making with respect to vaccine strategy. Further sequence analysis of the NA strains would be interesting to investigate their genetic relationship to Ark. Other pieces of information that were not provided for the analysis that could affect the results are the relationship each premise has with other premises, including ownership, shared equipment, and crews, and husbandry practices. If available, a much more robust model could be developed that would include the significance of geographic and nongeographic variables via a geographically weighted regression.

From a practical perspective, other infectious respiratory poultry diseases could be analyzed in a retrospective fashion in order to

Table 3. Spatial-temporal and spatial cluster fitted by a multinomial model for a given variant of IBV in California (2008–2012). The relative risk (RR) is reported for each genotype.

Category	Observed	Expected	Observed/Expected	RR
Spatial-temporal analysis				
Cluster 1, from 1/10/2009 to 2/3/2009[A]				
CA1737	4	0.52	7.71	n/a
Cal99	7	8.18	0.86	0.84
NA	0	5.58	0	0
Vacc	6	2.73	2.2	2.68
Cluster 2, from 3/20/2012 to 11/12/2012[B]				
CA1737	0	0.46	0	0
Cal99	0	7.21	0	0
NA	15	4.92	3.05	4.14
Vacc	0	2.4	0	0
Spatial analysis				
Cluster 1, from 8/1/2008 to 12/31/2008[C]				
CA1737	0	1.98	0	0
Cal99	28	31.26	0.9	0.81
NA	31	21.34	1.45	2.62
Vacc	6	10.42	0.58	0.41
Cluster 2, from 8/1/2008 to 12/31/2012[D]				
CA1737	4	1.56	2.57	n/a
Cal99	29	24.53	1.18	1.34
NA	5	16.74	0.3	0.21
Vacc	13	8.18	1.59	2.55

[A]$P < 0.01$; Number of producers in the cluster, broilers/layers = 122/2.
[B]$P < 0.01$; Number of producers in the cluster, broilers/layers = 41/6.
[C]$P = 0.069$; Number of producers in the cluster, broilers/layers = 108/41.
[D]$P < 0.01$; Number of producers in the cluster, broilers/layers = 106/4.

identify clusters of disease and advise the poultry industry about where biosecurity efforts should be focused in the future. In addition, the multinomial clustering models can be used to select appropriate IB vaccine types based on the genotypic risk ratios provided in the analysis (Table 3). Open source spatial analysis tools should be seen as complementary tools in order to better understand outbreaks, with the goal of using the combined data to mitigate outbreaks, focus surveillance efforts, and understand spatial-temporal transmission. In the future, at the state and university level, an extension of these types of analyses could be incorporated into infectious disease investigations in poultry in order to better inform relevant stakeholders about IB transmission and how best to respond.

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