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Spatial and Temporal Epidemiology of Infectious Laryngotracheitis in Central California: 2000–2012

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SUMMARY. In October of 2005 an outbreak of a vaccine-like strain of infectious laryngotracheitis (ILT), indistinguishable from the chicken embryo origin (CEO)-like vaccine strains, was detected by routine passive surveillance in the Central Valley of California, U. S. A. In response, a highly coordinated industry effort by two companies led to a significant decrease in the incidence of ILT over the same geographic region between 2008–2012. In order to understand the geographic and temporal spread of ILT in California before and after the outbreak, Global Information Systems (GIS) mapping coupled with spatial, temporal, and spatial-temporal statistics were used to identify retrospective and prospective low-rate clustering (i.e., less ILT than statistically expected) and high-rate clustering (i.e., more ILT than statistically expected) of ILT spatially and temporally. Results showed two high-rate retrospective spatial-temporal clusters and one low-rate prospective spatial-temporal cluster which were all statistically significant ($P < 0.05$). Overall, spatial-temporal clustering accounted for 36.9% of the positive ILT cases, while temporal clustering and spatial clustering done separately each accounted for 0% of the ILT cases, respectively. This demonstrates the utility of combining spatial and temporal clustering for ILT surveillance. Due to the risk of reversion to virulence and spread to immunologically naive broilers, future application of the CEO-based vaccine in the identified high rate spatial-temporal clusters should be avoided and other vaccine alternatives considered in order to avoid repeat outbreaks in those areas. This should especially be followed during the winter months of December, January, and February, which were found to have the highest prevalence of ILT ($P < 0.05$). Analysis of GIS data within the high-rate clusters showed that wind direction and farm density were minor factors in the spread of ILT. Shared roads may have played a role in the spread of ILT in one of the two high rate spatial-temporal clusters.

RESUMEN. Epidemiología espacial y temporal de laringotraqueitis infecciosa en el centro de California: 2000–2012.

En octubre del 2005 un brote de una cepa similar a la vacuna contra la laringotraqueítis infecciosa (ILT), indistinguible de las cepas vacunales originadas en embrión de pollo (CEO), fue detectada mediante vigilancia pasiva de rutina en el Valle Central de California, en los Estados Unidos. En respuesta, un esfuerzo altamente coordinado de la industria por dos empresas dio lugar a una disminución significativa en la incidencia de laringotraqueítis durante la misma región geográfica entre los años 2008–2012. A fin de comprender la distribución geográfica y temporal de la laringotraqueítis en California antes y después del brote, se utilizaron Sistemas de Información Global (GIS), junto con las estadísticas espaciales, temporales y espacio-temporales para identificar la agrupación espacial y temporal de tasa baja retrospectiva y prospectiva (es decir, menor que la tasa esperada estadísticamente para la laringotraqueítis) y de agrupamiento de tasa alta (es decir, mayor que la tasa esperada estadísticamente para laringotraqueítis) para laringotraqueítis. Los resultados mostraron dos agrupamientos espaciales-temporales retrospectivos de tasa alta y una agrupación espacial-temporal prospectiva de tasa baja que fueron estadísticamente significativas ($P < 0.05$). En general, la agrupación espacio-temporal representó el 36.9% de los casos positivos de laringotraqueítis, mientras que la agrupación temporal y la agrupación espacial por separado representó el 0% de los casos de laringotraqueítis, respectivamente. Esto demuestra la utilidad de la combinación de la agrupación espacial y temporal para la vigilancia de laringotraqueítis. Debido al riesgo de reversión a la virulencia y la diseminación a los pollos de engorde inmunológicamente susceptibles, la aplicación futura de vacunas basadas en CEO en agrupaciones espacio-temporales identificadas de tasa alta deben ser evitados y se deben considerar otras alternativas de vacunas a fin de evitar brotes repetidos en esas áreas. Esto sobre todo se debe seguir durante los meses de diciembre, enero y febrero, que se encontró que tenían la mayor prevalencia de laringotraqueítis ($P < 0.05$). Los análisis de datos de los sistemas de información global dentro de los grupos de alta tasa mostraron que la dirección del viento y la densidad de las granjas fueron factores de menor importancia en la difusión de la laringotraqueítis. Los caminos compartidos pudieron haber desempeñado un papel en la propagación de la laringotraqueítis en uno de los dos grupos espaciales-temporales de alta tasa.

Key words: ILT outbreak, GIS, spatial statistics

Abbreviations: CAHFS = California Animal Health and Food Safety System; C&D = cleaning and disinfection; CEO = chicken embryo origin; GIS = Global Information Systems; ILT = infectious laryngotracheitis; ILTV = infectious laryngotracheitis virus; OR = odds ratio; PCR-RFLP = PCR–restriction fragment length polymorphism

Infectious laryngotracheitis (ILT) is a highly contagious respiratory disease, primarily of chickens, caused by a *Gallid herpesvirus 1* (1). Chickens of all ages are susceptible and the disease is characterized by lacrimation, respiratory signs, and increased morbidity and mortality (6). Because treatments do not exist, the disease is primarily controlled by the implementation of biosecurity measures and vaccination (3). Although ILT was the first poultry pathogen controlled by vaccination, ILT still represents one of the most significant poultry diseases from a global industry perspective. The modified-live vaccine strains, and in particular the chicken-

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We dedicate this paper to our beloved friend and a co-author of the paper, Dr. Bruce Charlton, who passed away unexpectedly on May 26th 2014.

embryo-origin (CEO) strains used for vaccination in layers, have been identified as the source of several recent outbreaks in broiler chickens that are usually only vaccinated against ILT during an outbreak (2,11). When unvaccinated broilers are exposed to the CEO-like ILT from vaccinated chickens, they can develop a variety of adverse effects including ILT via reversion to virulence of the vaccine strain (5). The morbidity and mortality noted in unvaccinated broilers has led to the development of genetically manipulated vaccine strains (e.g., rHVT-LT [recombinant herpesvirus of turkeys vaccine expressing laryngotracheitis] and fowlpox-LT), which only express one gene of the ILTV genome and hence do not spread or revert to virulence. However, because these vaccines are more expensive relative to the conventional attenuated vaccines, they are used less often in the field.

Spatial, temporal, and spatial-temporal analyses of disease clustering are frequently used by epidemiologists to determine critical time-space boundaries for a disease and may be utilized in prevention and control programs (10). Clustering of disease in space, time, and space and time also provides clues to the factors which influence spread of the disease (10). ILT can be spread between farms in close vicinity by fomites and aerosols (2). An outbreak of vaccinal ILT started in 2005 in California and involved two main broiler companies situated in the Central Valley. Previous epidemiologic investigations in California suggested the source of this ILT outbreak to be either a vaccinated breeder flock 16 km (10 mi) from the index case or a contaminated truck moving live birds (4). In this study, we utilized statistically based Global Information Systems (GIS) clustering tools in order to identify statistically significant high-rate and low-rate clusters of disease in space, time, and space and time. In addition, by utilizing prospective cluster analysis, current high-rate or low-rate clustering can be detected. Furthermore, the effects of seasonality, farm density, and wind patterns on the prevalence of ILT were studied. The results can be used to make biosecurity and vaccine recommendations to farmers who are in geographic areas with high-rate ILT clustering.

MATERIALS AND METHODS

California Animal Health and Food Safety laboratory System (CAHFS) data between January 2000 and September 2012 were searched for broiler, layer, and backyard chicken cases or submissions that were diagnosed with ILT. Data on ILT-negative submissions between 2000 and 2005 were not included in the study because active surveillance for ILT was not started until the latter part of 2005, and inclusion of those ILT-negative results would have biased our analysis. ILT-positive data was based on gross and microscopic lesions and a combination of fluorescent antibody tests, PCR-restriction fragment length polymorphism (PCR-RFLP), immunohistochemistry, serology, and virus isolation, sequencing, or both. Additional data compiled by CAHFS included species, type of chickens tested for ILT (i.e., commercial broilers, layers, breeders, and backyard chickens), age of the birds, commercial companies involved, farms affected, month and year of occurrence of ILT, and vaccination and management history. Data were downloaded as a Microsoft Excel spreadsheet and imported into a Microsoft Access relational database (Microsoft Corporation, Redmond, WA).

Because the majority of the viruses identified in the field outbreaks were typed similar to the CEO vaccine virus, as determined by PCR-RFLP, for this paper the term ILT will be used instead of vaccinal aryngotracheitis (3). Cases for this study were defined as poultry premises that had diagnostically confirmed ILT as identified by the CAHFS laboratories. Controls for this study were identified as poultry premises that tested negative for ILT.

General spatial epidemiologic approach. In order to visualize the distribution of ILT-diagnosed cases, geocoding and geospatial mapping were conducted using the GIS program ArcGIS® v10 (ESRI, Redlands, CA; Fig. 1). For purposes of mapping, affected premises (i.e., farms) were defined as farms in which ILT was detected at least once in the flock's lifetime. Therefore, multiple positive tests from the same flock (i.e., group of birds on a farm housed in a separate house) were merged into a single accession or case. Consequently, a farm could be affected more than once if a separate or new flock was affected on the same farm that was previously identified as positive.

The following GIS layers within California were added: counties, roads, locations of poultry operations, locations of flocks that tested negative for ILT, locations of flocks that tested positive for ILT, and locations of flocks that had not been tested for ILT. The North American Datum 83 Projected Coordinate System and Geographical Coordinate System were used for mapping as part of ArcGIS® v10.

Spatial, temporal, and spatial-temporal cluster analysis. To investigate if any clustering of ILT occurred (defined as "global clustering"), a Global Moran I test with row standardization was applied to the geographic data, again using ArcGIS® v10. To investigate local clustering of ILT in space and time, statistical tests using a Bernoulli model were performed using SaTScan™ version 9.1.1 (National Cancer Institute, Bethesda, MD) (9). Likelihood functions were applied to various spatial windows in space or time to compare the number of observed and expected observations (i.e., observed number of cases within the cluster divided by the expected number of cases with the cluster) inside and outside the spatial or temporal window. The statistical significance of 'most likely clusters' (i.e., clusters that are least likely due to chance) was 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.

Combining the spatial and temporal analysis, spatial-temporal clustering imposes cylinders of varying size on the spatial-temporal data. The likelihood of ILT cases being located inside or outside of the cylinder was evaluated, and a risk ratio created and statistically evaluated using a Monte Carlo simulation under the null hypothesis of random distribution. A maximum window of 50% of the study area and a maximum temporal window of 1 yr of the study period were used. The test statistic of identified clusters was computed using a maximum likelihood ratio and the statistical significance of clusters was evaluated through Monte Carlo simulations (999 replications), and *P*-values <0.05 were considered statistically significant in order to reject the null hypothesis of random distribution in time and space. For all, temporal, and spatial-temporal analysis, data were analyzed both retrospectively (i.e., historic clusters) and prospectively (i.e., current or 'alive' clusters to allow for early detection of disease outbreaks). Any spatial cluster over 200 km in radius was not recorded.

In addition the spatial, temporal, and spatial-temporal data were analyzed as a single data set (i.e., the entire length of the study between December 2000 and December 2012) and stratified, where the data were split into two data sets (before and after August 2007).

Chi-square analysis, odds ratio (OR), and wind data. In order to determine the effect of seasonality (i.e., winter, spring, summer, and fall) on ILT prevalence, chi-square tests and their associated *P*-values were analyzed via the on-line tool MedCalc (www.medcalc.com). ORs and their associated *P*-values were used to compare the odds of ILT prevalence before and after the coordinated eradication response during the summer of 2007. Wind data was obtained from the National Climate Data Center, which is an on-line database and is part of the National Oceanic and Atmospheric Administration (NOAA). Data from three weather stations within the high-rate spatial-temporal retrospective cluster (Fig. 2A) were collected between December of 2006 and May of 2007. Utilizing wind patterns and the topography of the associated land, ILT spatial-temporal data were analyzed manually in order to assess if wind direction correlated with the temporal sequence of ILT cases within the clusters.

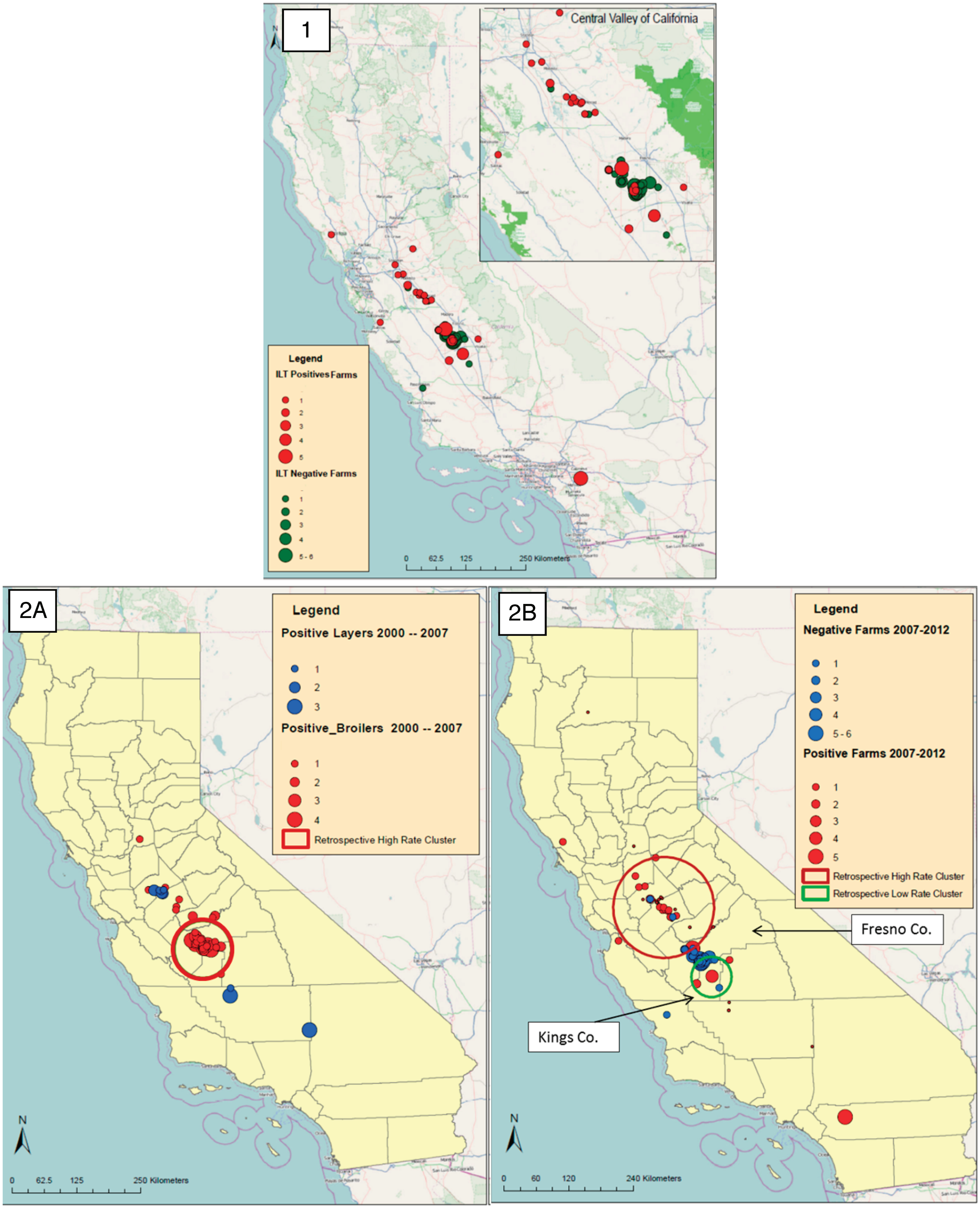

Fig. 1. Location of ILT-diagnosed layer and broiler farms between December of 2000 and December of 2012. Positive or negative farms refer to the number of positive or negative cases during the study period at that farm. Multiple positive or negative tests from the same flock were merged into a single positive or negative farm result.

Table 1. Descriptive statistics of data used for mapping and statistical analysis. These data only represent data that were mapped, which represents 91 separate locations. Data on ILT negative submissions between 2000 and 2005 were not included in the study.

Type	ILT-positive *n*	ILT-positive average age	ILT-negative *n*	ILT-negative average age	% Positive
Backyard	2	392 days	1	—	—
Broiler operations	115	35 days	103	37 days	—
Layer operations	24	38 weeks	NA[A]		—
2000	4		NA		NA
2001	2		NA		NA
2002	2		NA		NA
2003	1		NA		NA
2004	3		NA		NA
2005	13		NA		NA
2006	43		3		93
2007	45		36		55
2008	8		37		17
2009	5		20		20
2010	2		1		67
2011	1		1		50
2012	9		6		60
Totals	141		104		—

[A] NA = not applicable.

RESULTS

There were 422 accessions collected by the CAHFS laboratories in Fresno, Tulare, and Turlock, California. Accessions were selected based upon a flock signalment of clinical respiratory signs or flocks (or both) that were associated with an ILT case. Of those 422, 246 had address information. Of those 246, 141 accessions were confirmed positive for ILT based on gross and microscopic lesions and a combination of fluorescent antibody test, PCR-RFLP, immunohistochemistry, serology, virus isolation, and sequencing. Based on the above diagnostic tests, the remaining 105 accessions were confirmed to be negative for ILT. A map of these positive and negative farms was generated in order to show the distribution of ILT during the study period (Fig. 1). Based on interviews with company veterinarians and ranch managers, at the onset of the initial outbreaks in October of 2005 most of these flocks had not been vaccinated for ILT but had been raised in the vicinity of layer flocks or broiler breeders (or both) which were vaccinated with a CEO-like vaccine. Although layer, breeder, and backyard chicken flocks were included in this study, 81.5% of the ILT-positive flocks were broilers (Table 1). The age of the broiler chickens submitted ranged between 18 and 49 days with an average of 35 days (Table 1). Of the cases occurring between 2000 and 2012, 62.4% of the ILT cases were identified between 2006 and 2007 (Table 1).

Grouping all the positive data based on seasonality, a one-way Chi-square test showed that, over the length of the study, we can reject the null hypothesis that there were no differences between the prevalence of ILT based on seasonality ($P < 0.005$). The winter months of December, January, and February had the highest prevalence of ILT, with 54 different premises affected during the entire length of the study, as opposed to the spring, summer, and fall which had 37, 25, and 25 affected premises affected, respectively, over the entire study period (Fig. 3).

Starting in August of 2007 an extended downtime on each positive farm, coupled with a new vaccine protocol, was instituted (3). Based upon this observation, cluster analysis was done either without any stratification (i.e., the entire length of the study between October 2000 and December 2012) or stratified, where the data were split into two data sets (before and after August 2007). An OR showed that farms were 49.2 times more likely to have CEO-like ILT before August of 2007 than after August of 2007 ($P < 0.05$; Fig. 3). When looking at the data without any stratification by date, a low-rate (e.g., less disease than statistically expected) temporal cluster was observed between August of 2007 and August of 2009 (Table 2). Specifically, 12 cases were observed in that temporal window while 57.89 cases were expected (Table 2). This coincided with the implementation of extended down time, increased cleaning and disinfection (C&D), and new vaccine protocols, all of which were implemented in August of 2007 in order to stop the ILT outbreak. In addition, a low-rate prospective (e.g., on-going) spatial-temporal cluster was observed between 2007 and 2012 in Fresno County, California. In this low-rate spatial-temporal cluster, only 4 cases were observed while 44.13 were expected.

When stratifying the data by pre-August 2007 and post-August 2007, one low-rate and two high-rate spatial-temporal clusters were identified (Table 3) One of the high-rate clusters was a retrospective spatial-temporal cluster in Fresno County between October 2006 and May 2007 that accounted for 36.4% of all the ILT-positive data between the start of the study and August of 2007 (Fig. 2A; Table 3). Overall the two high-rate spatial-temporal clusters accounted for 52 of the positive cases or 36.9% of all the positives in the entire study (Table 3). The low-rate spatial-temporal cluster overlapped geographically with the high-rate spatial-temporal cluster in Fresno and Kings counties (Table 3).

In order to determine the role that farm density played in ILT transmission, the area of the low-rate and high-rate spatial and

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Fig. 2. (A) Location and quantity of CEO-like ILT-positive broiler and layer farms between December of 2000 and August of 2007. The high-rate spatial-temporal cluster includes 40 of the 46 positive CEO-like ILT-positive farms identified between October of 2006 and May of 2007 (Table 2). (B) Location of CEO-like ILT-positive and negative broiler and layer farms between September of 2007 and December of 2012. One retrospective low-rate and retrospective high-rate spatial-temporal cluster are identified.

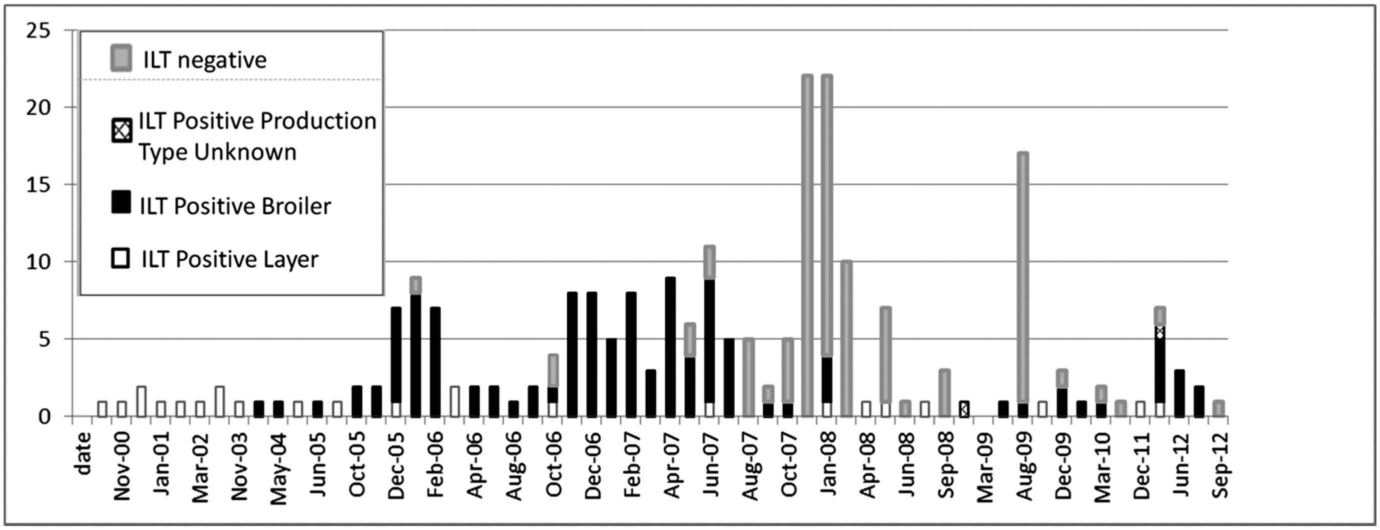

Fig. 3. Histogram of the number of ILT-positive ($n = 141$) and negative ($n = 103$) test results submitted to the CAHFS labs between September of 2000 and September of 2012. An OR showed that farms were 49.2 times more likely to have CEO-like ILT before August of 2007 than after August of 2007 ($P < 0.05$). The winter months of December, January, and February were found to have the highest prevalence of ILT ($P < 0.05$). A case was defined as a premise that was affected with ILT once in a flock's life. Data on ILT-negative submissions between 2000 and 2005 were not included in the study.

spatial-temporal clusters was calculated and divided by the total number of poultry operations (data not shown) present in that area. The resultant value referred to as the circular area per number of premises did not show any consistent observations with respect to the relationship between ILT prevalence and the density of poultry operations (Tables 2, 3).

When investigating the ILT farms within the high-rate retrospective cluster identified in Figure 2A, the first two cases were separated by 1 day and 3.42 mi (the second case was due east of the first case). Both premises were owned by the same company. The third case occurred 1 mo later and was 2.83 mi to the west of the first case. The fourth case occurred 4 days after the third case and occurred 15.38 mi west of the third case. The fifth and sixth cases occurred 18 days after the fourth case. The fifth and sixth farms are less than 1.2 mi west of the fourth case. The third through 20th cases in this time-space cluster were all owned by the same broiler company. These 20 cases occurred in a 4-mo period of time. When assessing historic wind patterns from three weather stations within this high-rate cluster (Fresno Air Terminal, Hanford Muni, and Lemoore), the wind direction from all the stations moved primarily in a northwesterly direction with wind speeds ranging between 0–19 mi/hr. Consequently, based on wind direction, the flat topography of the associated land, and the temporal pattern of ILT positivity submitted to the CAHFS lab, five cases could have been spread by wind.

DISCUSSION

Because the strain of ILT identified in all the cases that were characterized was CEO-like, the spread of the virus from one farm to another farm was most likely related to the transmission of the virus via aerosols and fomites. Specifically, transmission by employees, contract employees, and companies with multiple premises that share equipment, roads, and wind vectors from clinically positive ILT cases may all have contributed to the spread of ILT (5,8). Consequently, this type of continuously propagating outbreak would lend itself to spatial-temporal cluster analysis. Our results showed that spatial-temporal clustering accounted for 36.9% of the positive ILT cases while temporal clustering and spatial clustering done separately each accounted for 0% of the ILT cases, respectively. (Tables 2, 3). This demonstrates the utility of combining spatial and temporal clustering for ILT surveillance. Further interventions can be focused within these high-rate clusters to prevent future outbreaks as opposed to carrying out the same interventions where no-risk or low-rate clustering was observed (Tables 2, 3).

Between December 2000 and July 2007, 110 positive ILT accessions were observed in California. In August 2007 new protocols were implemented, including extended down time between flock placements (e.g., 30–91 days), increased biosecurity and C&D, enhanced surveillance, and a new vaccine protocol (3). These disease-mitigation protocols were discontinued after February of 2008 due to their apparent (i.e. outbreaks can end naturally without any intervention) success in reducing the incidence of ILT. Specifically, before August 2007 the prevalence of CEO-like ILT was 49.2 times greater than after August 2007 ($P < 0.05$). In addition, the low-rate retrospective spatial-temporal cluster between August 2007 and December 2012 overlapped spatially with the high-rate retrospective spatial-temporal cluster between October 2007 and September 2008, thus providing further evidence supporting the efficacy of the intervention strategies practiced from August 2007 to February 2008 (Fig. 2A,B).

Layers and broiler breeders are routinely vaccinated with CEO vaccine strains that are known to cause a variety of adverse effects including spread of the vaccine virus to nonvaccinated individuals, insufficient attenuation, production of latently infected carriers, and increased virulence as a result of *in vivo* (bird-to-bird) passages (1,7). At the onset of the initial outbreaks in this study, most of the broiler flocks had not been vaccinated for ILT but were raised in the vicinity of broiler breeders and, in some cases, layers which were vaccinated with a CEO-like vaccine. Interestingly, following the implementation of control practices against ILT a high-rate retrospective cluster was identified in December of 2009 (Table 3). Seven layer operations were noted in the geographic area encompassing this spatial-temporal cluster. This is in contrast to four layer operations noted on the outskirts of the high-rate cluster in Fresno and Kings counties (Table 3). Broiler breeder facilities were not specifically identified in our database.

Table 2. Statistically significant retrospective temporal and prospective spatial-temporal low rate cluster analysis of CEO-like ILT in California poultry flocks from 2000–2012 using the Bernoulli model. No stratification of the data was used. The relative risk is the estimated risk within the cluster divided by estimated risk outside the cluster (8).

Location	Type of cluster	Time period	Radius (km)	Area (km)	Circular area/number of premises	Observed cases in cluster	Expected cases in cluster	Ratio (observed / expected)	Relative risk	*P*-value
Fresno County	Low-rate prospective spatial-temporal	10/1/2007–11/30/2012	15.76	780.30	19.51	4	44.13	.091	.064	.001
NA[A]	Low-rate retrospective temporal	—	NA	NA	NA	12	57.89	0.21	.13	.001

[A] NA = not applicable.

Table 3. Statistically significant retrospective spatial-temporal low rate or high rate cluster of ILT in California poultry flocks between 2000 and August 2007 and between September 2007 and December of 2012 using the Bernoulli model. These data were initially stratified into two categories; samples before August 2007 and samples after August 2007. The relative risk is the estimated risk within the cluster divided by estimated risk outside the cluster (8).

Location	Type of cluster	Time period	Radius (km)	Area (km)	Circular area/number of premises	Observed cases in cluster	Expected cases in cluster	Ratio (observed /expected)	Relative risk	*P*-value
Fresno and Kings counties	High rate retrospective spatial-temporal	10/2006–5/2007	55.44	9651.08	74.81	40	20.47	1.95	2.50	<0.05
Fresno and Kings counties	Low rate retrospective spatial-temporal	10/2007–9/2008	37.35	4380.37	91.26	2	14	0.14	0.077	<0.05
Primarily Merced and Stanislaus counties	High rate retrospective spatial-temporal	12/2009–7/2012	93.56	27485.91	68.71	12	3.12	3.84	5.64	<0.05

Our results show no apparent correlation between overall poultry farm density and likelihood of ILT-positive flocks (Tables 2, 3). This may be due to the statistical area that was constructed for the density calculation (i.e., the circular windows). To fully assess the significance of poultry farm density and layer-to-broiler ratios as risk factors, more appropriate polygons and grids with Hot Spot Analysis (ArcGIS® v10.2) should be constructed.

The ILT virus can be spread by the transportation of animals, personnel, and equipment. One of the critical points identified as a potential source of virus transmission was roads that were frequently used by the poultry industry within the outbreak area. As a biosecurity measure, companies required their personnel to use specific routes with either "clean" or "dirty" roads to avoid other facilities or ranches (3). At a larger regional scale, within the high-rate clusters it is difficult to assess whether roads are a significant factor in ILT dissemination. Further magnification (not shown) of the outbreak maps in Figures 2 and 3 shows smaller roads that are most likely used by multiple farms within the high-rate clusters. Hence the possibility of dissemination of virus via transportation is possible. Between December 2009 and July 2012, one significant high-rate retrospective spatial-temporal cluster was observed (Table 3). These premises are primarily north of Fresno County (Fig. 2B). Interestingly, four of the affected broiler farms which submitted samples within a 2-day period area are all owned by the same company. Three of the four are located off the same road (data not shown). It could be supposed that there was some type of break in biosecurity. Previous work has shown that "live-haul" trucks transporting birds with ILT are likely to spread the infection to susceptible birds that are close to the road (5). In addition, poor biosecurity with respect to dead bird and manure disposal has also been identified as a mode of indirect transmission of ILT (5). Further study, perhaps using GPS devices on farm trucks, would be helpful in determining the significance of these biosecurity issues. It is also important to consider that the commercial farms included in this study are scattered among the ever-increasing number of backyard poultry premises, feed-stores, auctions, and farmers markets that are present in urban-rural interfaces such as the Central Valley of California. Capturing and including this data in future analyses could offer new clues for disease transmission.

Based on the wind data and the flat topography of the land, wind may have played a minor role (i.e., 5 of the first 20 cases in the high-rate cluster identified in Figure 2A) in the transmission of ILT within the high-rate clusters (Fig. 2A). However, when assessing the impact of wind it is difficult to determine the significance of the data. A northwest wind traveling between two farms, which shows the farm further east getting ILT after the first farm within a time period that would account for both virus dissemination and infection, would provide circumstantial evidence of disease spread via wind. Several cases, including the second and third cases in the high-rate cluster in Fresno and Kings counties (Table 3), fit within these parameters. However other factors, including shared roads and breakdowns in biosecurity due to joint ownership (the majority of the cases were from 2 separate farms), may also have played a role in disease transmission. Johnson *et al.* (8), using a GIS-based program that generates vectors representing wind patterns, found wind to be a significant risk factor. In order to reduce the risk of airborne transmission for high-risk farms, managers may want to consider reimplementation of the interventions strategies described by Chin *et al.* (12) and, potentially, further tools including installation of air scrubbers, changes in house ventilation rates, and ionization systems that have been shown to reduce dust concentrations; all could be an efficient way to stop infectious particles from getting in or out.

In addition to the factors described above, the prevalence of ILT was shown to be statistically different based on seasonality. Over the length of the study, the winter months of December, January, and February had the highest prevalence of ILT (54 total cases) while the warmer summer and fall months had a lower prevalence (25 cases for each). Previous work has demonstrated the sensitivity of the ILT virus to be activated by both light and heat (5).

The first step in controlling the spread of a disease is to understand the relationship between the agent, host, and the environment. An understanding of this classic epidemiologic triad is essential toward breaking the transmission cycle of any infectious disease. Previous studies related to this outbreak have demonstrated that the most effective way to prevent or control an ILT outbreak is through enhanced biosecurity, C&D, and implementation of an appropriate vaccination program (3). In this study we use GIS and spatial statistics to demonstrate the potential of spatial-temporal clustering as a tool for retrospectively and prospectively identifying outbreaks of ILT. Specifically, spatial-temporal clustering accounted for 36.9% of the ILT cases while spatial and temporal clustering accounted for 0% of the ILT cases, respectively. The remaining 63.1% of the cases did not have spatial-temporal clustering. However, it should be noted that before the new biosecurity protocols were implemented in August of 2007, 52 of the 110 ILT cases (47.3%) were associated with spatial-temporal clustering. Therefore, spatial-temporal clustering of ILT appeared to be more likely before the new biosecurity protocols were implemented than after.

Once the parameters of these spatial-temporal clusters are identified, control practices should be focused within these high-risk areas (i.e., high-rate clusters) in order to focus resources to avoid future outbreaks in the highest risk areas. Based on our interpretation of the data, farm density and wind do not appear to play a significant role in the spread of ILT. However, due to the knowledge of how the CEO-like ILT vaccine is given (e.g., broiler breeders and layers), we would recommend the adoption of a non–CEO-like ILT vaccine within the retrospective high-rate clusters in order to prevent the spread of CEO-like ILT to immunologically naive broilers. Furthermore, prospective clustering, a clustering tool used for the early detection of disease outbreaks, should be used in a continuous fashion in order to identify new outbreaks (9). Epidemiologic tools, including GIS and spatial statistics, are essential toward understanding risk and how diseases such as ILT may spread based on the local and regional proximity of poultry operations. Clustering of disease events in time, space, and space and time provides clues to the epidemiology of ILT and hence may assist in prevention and control programs.

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