AVIAN DISEASES 57:76–82, 2013

Historical, Spatial, Temporal, and Time-Space Epidemiology of Very Virulent Infectious Bursal Disease in California: A Retrospective Study 2008–2011

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Received 16 July 2012; Accepted 21 October 2012; Published ahead of print 22 October 2012

SUMMARY. In December of 2008 very virulent infectious bursal disease virus (vvIBDV) was identified in a commercial flock in northern California. Since then several other backyard and commercial facilities in California have had flocks affected by the same strain and other unique (previously unseen) strains of IBDV. Previous to this incident, very virulent infectious bursal disease (vvIBD) had never been identified in North America. Following the initial outbreak in 2008, California became the first state to undertake a voluntary surveillance effort to try to determine the geographical prevalence of vvIBD based on sequencing of a portion of the segment A region of the vvIBDV genome. To date we have complete geographical information on approximately 500 separate accessions representing approximately 1500 birds from over 200 commercial (~85% of the facilities) and backyard facilities (~15% of the facilities) throughout the state. Sequencing of targeted regions of both the segment A and segment B regions of the genome has revealed three distinct types of IBDV in California chickens. One type is genetically and in pathogenically consistent with vvIBDV. The second and third types only have a segment A region consistent with vvIBDV. Geographic information system mapping coupled with spatial-temporal cluster analysis identified significant spatial and time-space clustering; however, no temporal clustering was noted. The lack of temporal clustering coupled with negative vvIBDV results in tested avian wildlife implies that avian wildlife in California do not currently appear to play a significant role in vvIBDV transmission. In the voluntary surveillance that was done in the Central Valley of California, which has a high density of commercial poultry, no positive farms were found when 142 of 504 farms were sampled. Given this level of sampling, the confidence (probability) of detecting an affected commercial flock was calculated to be between 28% and 81% depending on whether one or five hypothetically affected farms were affected.

RESUMEN. Epidemiología histórica, espacial y espacio-temporal del virus muy virulento de la enfermedad infecciosa de la bolsa en California: Un estudio retrospectivo 2008–2011.

En diciembre del 2008, se identificó un virus muy virulento de la enfermedad infecciosa de la bolsa (con las siglas en inglés vvIBDV) en un lote comercial en el norte de California. Desde entonces, varias instalaciones avícolas comerciales y de traspatio en California han tenido parvadas afectadas por la misma cepa y otra cepa única (no detectada anteriormente) del virus de Gumboro. Antes de este incidente, el virus muy virulento de la enfermedad infecciosa de la bolsa nunca había sido identificado en América del Norte. Después del brote inicial en el año 2008, California se convirtió en el primer estado en realizar un esfuerzo voluntario de vigilancia para determinar la prevalencia geográfica del virus muy virulento de Gumboro basado en el análisis de secuencias de una porción del segmento A del genoma de este virus muy virulento. Hasta la fecha se cuenta con información geográfica completa de aproximadamente 500 registros de diagnóstico diferentes que representan aproximadamente 1,500 aves de más de 200 instalaciones comerciales (aproximadamente el 85% de las instalaciones) y de las instalaciones de traspatio (aproximadamente el 15% de las instalaciones) en todo el estado. El análisis de las secuencias de las regiones blanco de los segmentos A y B del genoma ha revelado tres tipos distintos de cepas del virus de Gumboro en pollos de California. Un tipo es genética y patogénicamente similar con el virus muy virulento de Gumboro. El segundo y tercer tipos solo tienen un segmento A similar a las cepas virulentas. El mapeo por sistemas de información geográfica (con las siglas en inglés GIS), junto con el análisis de conglomerados espacio-temporales identificaron agrupamientos espaciales y espacio-temporales significativos, sin embargo, no se observó agrupación temporal. La falta de agrupación temporal junto con los resultados negativos para la presencia de cepas muy virulentas en la fauna silvestre aviar implica que esta fauna silvestre de California no parece jugar un papel importante en la transmisión de las cepas muy virulentas del virus de Gumboro. En la vigilancia voluntaria que se llevó a cabo en el Valle Central de California, que tiene una alta densidad de avicultura comercial, no se detectaron granjas positivas cuando se muestrearon 142 granjas de un total de 504. De acuerdo a este nivel de muestreo, la confianza (probabilidad) de detección de una parvada afectada se calculó entre 28% y 81% dependiendo de si una o cinco granjas afectadas hipotéticamente resultaron afectadas.

Key words: vvIBDV, IBD, poultry, temporal spatial clustering

Abbreviations: BY = backyard; CAHFS = California Animal Health and Food Safety Laboratory; GIS = geographic information system; IBD = infectious bursal disease; IBDV = infectious bursal disease virus; OIE = World Organisation for Animal Health; SPF = specific pathogen free; vvIBD = very virulent infectious bursal disease; vvIBDV = very virulent infectious bursal disease virus

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Infectious bursal disease (IBD), also known as Gumboro disease, is an acute, contagious, viral infection that causes subclinical immunosuppression in susceptible chickens 3 wk of age or younger and clinical disease in susceptible older birds (18). The disease is characterized by increased mortality, inflammation and atrophy of the bursa of Fabricius, and hemorrhages in skeletal muscle (18). IBD is one of the major causes of economic loss in young chickens due to morbidity, mortality, and immunosuppression (18). IBD virus (IBDV) is a member of the genus *Avibirnavirus* of the family *Birnaviridae* and consists of two double-stranded RNA segments, designated A and B (6). IBD is an acute and contagious disease in young chickens that targets immature B lymphocytes in the bursa of Fabricius (18). Specifically, IBDV replicates in developing B lymphocytes and compromises both humoral and cellular immune responses (17).

Two distinct serotypes of IBDV have been recognized worldwide (2). Serotype 1 infects and causes clinical disease in chickens, while serotype 2 viruses are infectious but nonpathogenic to turkeys (2). Strains of serotype 1 include classic or standard IBDV, very virulent IBDV (vvIBDV), antigenic variant IBDV, and attenuated IBDV (12,13). The strains are typically classified based on phenotypic and/or genetic traits (2). In particular, vvIBDV is associated with a more severe disease causing high mortality in susceptible chickens (13). Mortality rates of 100% have been reported in experimentally infected specific pathogen free (SPF) chickens. Mortality rates of 60% in layer pullets and 30% in broilers have been reported in field outbreaks with vvIBD (2).

A wide range of IBDV serotype 1 genotypes exist in nature. Previous studies by Jackwood *et al*. (9), identified up to six different types of IBDVs based on sequence analysis of the hypervariable region of the VP2 gene within segment A of the IBDV genome in the United States. Another recent study identified reassorted IBDV from two premises in Northern California (11). While the segment A of the reassortments was typical of vvIBDV, the segment B was most similar to IBDV serotype 2 (11). Specifically, regions of these reassortments of genome segment B appear to have significant diagnostic value with respect to identifying vvIBDV *vs.* non-vvIBDV subtypes (1,7). In addition, these novel reassortments affect the mortality that the virus causes as detected by laboratory infections in SPF birds (11). While there have been previous reports of natural reassortments between vvIBDV and other serotype 1 strains, it is not known how many divergent strains of IBDV exist or how they may be distributed geographically, either internationally or within the United States (9,11).

Since vvIBDV was first diagnosed in the Netherlands in 1987 (8), it has spread to some parts of Latin America, Asia, Africa, and most recently, in December 2008, to Northern California (10,11,19). Like classical IBDV, vvIBDV can spread quickly through flocks, with an incubation time and excretion onset between 1 and 3 days (8). Infected chickens can continue to excrete the virus in their feces for up to 2 wk following infection (8). Once established on a farm, the virus is extremely persistent and difficult to eliminate through routine cleaning and disinfection (6). Specifically, the virus has been shown to remain infectious for 122 days in a chicken house and for 52 days in feed, water, and feces (8).

Spatial and temporal analyses of disease clustering are frequently used by epidemiologists to determine critical time-space boundaries for a disease. These tests allow the differentiation of statistically significant disease outbreaks from cases of disease due to chance, and they facilitate the creation of effective containment strategies (15). In this study, we present a redacted historical (2008–2011) and epidemiological summary of the initial detection of multiple IBDV subtypes between 2008 and 2011. Additionally, spatial, temporal, and time-space analyses were used to identify and characterize clusters of positive flocks in time and space. Finally, a statistical analysis was performed to assess the likelihood that the Central Valley, the region of California with the highest concentration of poultry, is free from vvIBD.

MATERIALS AND METHODS

Historical analyses of the vvIBD outbreak and mapping. During the initial outbreak of vvIBD, private veterinarians and flock managers who suspected vvIBDV in their flock(s) submitted live birds, carcasses, or both to the California Animal Health and Food Safety Laboratory (CAHFS) for reverse-transcriptase PCR of the segment A region and/or virus isolation of lymphatic tissue(s). Sequencing of the segment B region was only attempted if the segment A region was consistent with vvIBDV. In most cases, if vvIBDV segment A was identified as vvIBDV compatible, veterinarians from the California Department of Food and Agriculture and the United States Department of Agriculture Animal Plant Health Inspection Service visited affected backyard (BY) and commercial facilities to help evaluate risk factors and identify the source of the disease. For purposes of this study, a positive vvIBD case was defined as a premise that was affected with a vvIBDV segment A compatible sequence once in the flock's life. Therefore, multiple positive tests from the same flock were merged into a single accession or case. Consequently, a premise could be affected more than once if a separate or new flock was affected on the same premise that was previously identified as positive.

General spatial epidemiological approach. Available data from CAHFS was downloaded as a Microsoft Excel spreadsheet and imported into a Microsoft Access relational database. Geographic information system (GIS) mapping was conducted using ArcGIS® v10, to visualize statewide surveillance efforts and the scope and distribution of vvIBD cases in the state. A IBDV subtype was defined as *negative* if the segment A was not consistent with vvIBDV; *vvIBDV/vvIBDV* if the segment A and segment B sequences were consistent with vvIBDV; *vvIBDV/standard* if the segment A was consistent with vvIBDV and the segment B was consistent with a standard IBDV sequence; and *vvIBDV/serotype 2* if the segment A was consistent with vvIBDV and the segment B had a sequence consistent with a re-assortment with serotype 2 IBDV segment B. For purposes of the GIS analysis, affected farms were defined as farms in which the described sequences were present at least once. Two presumptive positive cases were unable to be subtyped (vvIBDV/*indeterminate*) and were excluded from the analysis.

Using ArcGIS, we combined a map of California with the locations of commercial and BY poultry operations to visualize the geospatial and temporal distribution of vvIBD and to identify areas of large-scale poultry production that had not yet been tested for vvIBDV. We created the following layers within California: counties, locations of poultry operations, locations of flocks that tested negative for vvIBDV, locations of flocks that tested positive for vvIBDV segment A, and locations of flocks that had not been tested for vvIBDV.

To investigate local clustering of vvIBDV and other IBDV subtypes in California poultry flocks and clustering in time and space, spatial, temporal, and time-space statistical tests were performed using SaTScan™ version 9.1.1 (National Cancer Institute, Bethesda, MD).

Spatial and temporal cluster analysis. Cluster analyses were conducted using SaTScan. Spatial cluster analysis imposes circles of varying size on spatial data to identify significant clustering of cases. For each location and size of the circle, a likelihood ratio of a case flock within and outside of the circle is generated and statistically evaluated using a Monte Carlo simulation (3). The window size and location least likely to be due to chance is the most likely cluster, with non-overlapping secondary clusters ranked based on *P* value. The case-control vvIBDV subtype data were tested using a Bernoulli approach with a maximum window of 50% of the study area, with 999 Monte Carlo replications of the data, and *P* value. The temporal scan statistic

uses a window that moves in a single dimension, time, with the height of the window representing the time period of potential clusters (14). The likelihood of each vvIBDV subtype flock falling inside or outside of the window was statistically evaluated using a Monte Carlo simulation under the null hypothesis of random distribution, with 999 Monte Carlo replications of the data, and cluster with P values ≤ 0.05 were considered statistically significant.

Space-time scan statistic. SaTScan was used to evaluate local (regional within California) time-space clustering (15). Similar to the spatial scan, the space-time permutation imposes cylinders of varying size on the spatial-temporal data. The likelihood of a vvIBD case flock 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. Cluster assessment was done by comparing observed positives to expected positives given a random distribution in space and time. The test statistic of identified clusters was computed using a maximum likelihood ratio, and the P values were determined by Monte Carlo simulation with 999 replications of the data under the null hypothesis. The most likely primary cluster and non-overlapping secondary clusters were considered significant if they had a P value ≤ 0.05.

Statistical evaluation of sampling. In order to determine the risk of vvIBD in the Central Valley, surveillance data was evaluated in the context described by the World Organisation for Animal Health (OIE) with respect to demonstrating freedom from infection. Specifically per the OIE, confidence is the probability that the type of surveillance applied would detect the presence of infection if the population were infected (16). Using the OIE's description, the surveillance data were evaluated using a hypergeometric approximation (4): $P(r = 0) = \{[M - n - (x/2) + 0.5]/[M - (x/2) + 0.5]\}^x$, where $x = p_s D + q_e(M - D)$ and n = the sample size, r = the number of farms responding positively via diagnostic testing, $P(r = 0)$ is the probability that the number of farms responding positively will be zero, D = the number of farms in which the disease agent is actually present, M = the total population of farms being considered, p_s = the farm level sensitivity of the diagnostic testing, and $q_e = 1 -$ the farm level specificity of the diagnostic testing.

It was assumed that the specificity of the diagnostic testing for vvIBDV segment A was 100%. The basis of this assumption is that no flocks responding positively to the diagnostic testing have been determined to be false positives. The farm-level sensitivity of the diagnostic testing is unknown, but for this analysis it was assumed to be 100% to represent a best case scenario for the diagnostic testing. Likewise, as a best case scenario, it was assumed that the voluntary surveillance was representative of the farms in the Central Valley and that if there was vvIBD, the distribution was not clustered (in other words, not biased compared to a random sample).

RESULTS

Descriptive epidemiology. Table 1 provides a redacted history of known cases of IBDV subtypes in California between December of 2008 and December of 2011. Of the 25 cases, 19 cases were in commercial facilities and six were in BY facilities. Of the 19 commercial facilities, 13 of them came from ranches owned by a single broiler facility.

Spatial results. Data were available for 486 submissions over a period of 36 mo. Each submission represented one unique poultry premise or a repeat submission from a premise, if a different flock was affected. The highest frequency of vvIBD cases occurred in 2010 and 2011, with 12 and 11 cases, respectively. The month with the highest frequency of cases was August 2011, with five cases (Fig. 1). Maps of positive and negative flocks showed a high concentration of positive flocks in Northern California (Fig. 2a). Two geographically distinct occurrences of subtypes of IBDV occurred outside of northern California. The first was diagnosed as vvIBVD/vvIBDV, and occurred in northeastern California in September 2010. The second incident occurred in southern California during September and October 2010 and involved the vvIBD/standard subtype. As of December 2011, neither vvIBDV nor any subtypes of IBDV had been diagnosed in the Central Valley of California, the state region with the highest concentration of commercial poultry farms (Fig. 2b).

Spatial clustering of vvIBD was detected using SaTScan. The Bernoulli model for the spatial scan detected one significant spatial cluster of vvIBDV/vvIBDV. The cluster, which included 22 submissions, was centered in northern California and had a radius of 30.6 km ($P \leq 0.001$; Table 2). No significant temporal clustering of vvIBDV/vvIBDV was detected. One significant time-space cluster of vvIBDV/vvIBDV was detected using the space-time scan. The cluster of four cases was centered in northern California from August 18, 2010, to October 28, 2011, and had a radius of 1.64 km ($P = 0.012$; Table 3).

vvIBDV/standard was detected in one pullet facility in Southern California. No statistically significant clustering was noted. One significant spatial cluster of vvIBDV/serotype 2 was detected using the Bernoulli model for the spatial scan. The cluster contained seven submissions and was centered in northern California, with a radius of 35.8 km ($P \leq 0.001$; Table 2). No significant temporal or time-space clusters of vvIBDV/serotype 2 were detected.

Statistical analysis to demonstrate freedom from vvIBD in the Central Valley of California. From the voluntary surveillance completed between 2008 and 2011, the data showed that 142 of the 504 commercial poultry farms in the Central Valley had been sampled since the beginning of the outbreak in December 2008. Due to the large concentration of poultry in the Central Valley, we completed an analysis to determine the statistical likelihood of the Central Valley being free from vvIBD. For this analysis, two hypothetical scenarios were analyzed in order to determine the probability that the counties of Tulare, San Joaquin, Merced, Fresno, and Stanislaus in the Central Valley of California were free from vvIBD. In the first hypothetical scenario only 1 of the 504 commercial farms had vvIBDV (based on the segment A sequence) present. In the second hypothetical scenario 5 of the 504 farms had vvIBDV present. For the first scenario (0.2% hypothetical prevalence, using one farm as the threshold), the probability that the affected flock would be 1 of the 142 flocks sampled is 28% (conversely the probability that the affected flock would not be selected is 72%). For the second scenario (~1% hypothetical prevalence, using five farms as the threshold), the probability of detecting at least one affected flock is 81%. Consequently, per the OIE definition of "confidence," coupled with the hypothetical prevalence levels selected, the confidence that the Central Valley is free of the vvIBD was between 28% and 81%, depending on the detection threshold used.

DISCUSSION

Results of the clustering tests demonstrate that statistically significant spatial clustering of two subtypes of vvIBDV—vvIBDV/vvIBDV and vvIBDV/serotype 2—occurred in California during the study period, 2008 to 2011. The results of the spatial scans indicated that for these specific locations the number of diagnosed cases of each subtype was greater than what would have been expected by chance (Table 2). These results provide statistical support for the geographical observation that cases of vvIBDV/vvIBDV and vvIBDV/serotype 2 appeared to be clustered in

Table 1. Redacted historical description of significant vvIBDV cases and subtypes in California between December 2008 and 2011.

Case #	Date	Commercial/BY	Production class	IBDV subtype	Distance from index case (km)	Approximate age (wk)	IBD vaccine status	Reported mortality	Miscellaneous
1	Dec. 2008	Commercial	Layer	vvIBDV/vvIBDV	—	10	Vaccinated[A]	5%–30%	—
2	Dec. 2008	Commercial	Layer	vvIBDV/vvIBDV	4.0	Unknown	Unknown	~30%	[B]
3	May 2009	Commercial	Brooding facility	vvIBDV/serotype 2	4.0	5, 7	Vaccinated[C]	~15	[D,E]
4	May 2009	Commercial	Broiler	vvIBDV/vvIBDV	3.2	3	Vaccinated	<1%	—
5	Aug. 2009	Commercial	Broiler	vvIBDV/vvIBDV	6.4	4	Vaccinated	<1%	[F]
6	Oct 2009	Backyard	Layer	vvIBDV/serotype 2	51.5	28	Unknown	Unknown	—
7	Jan. 2010	Commercial	Broiler	vvIBDV/vvIBDV	22.5	3.5	Not vaccinated	<1%	[G]
8	Jan. 2010	Backyard	Layer	vvIBDV/vvIBDV	8.9	10–11	Unknown	~15%	[H]
9	June 2010	Backyard	Layer	vvIBDV/[I]	1.6	10–12	Unknown	Unknown	—
10	Sept. 2010	Backyard	Unknown	vvIBDV/vvIBDV	226.9	16	Unknown	Unknown	—
11	Oct. 2010	Commercial	Brooding facility	vvIBDV/standard	[J]	1–10	[K]	[L]	—
12	Dec. 2010	Commercial	Brooding facility	vvIBDV/[M]		5, 7	Vaccinated	~5%	—
13–23	Jan. 2011 and present	Commercial	Broiler	vvIBDV/vvIBDV or vvIBDV/serotype 2	[N]	~4, younger	Vaccinated	<1%	—
24	Aug. 2011	Backyard	Layer	vvIBDV/serotype 2	30.6	4	Not vaccinated	Unknown	—
25	Sept. 2011	Backyard	Layer	vvIBDV/[I]	30.6	Unknown	Not vaccinated	Unknown	—

[A]Owner said that he gave vaccine via a fog-type application method just prior to the mortality. However, titer values of the birds in the affected house with the highest mortality showed no evidence of vaccination.
[B]It was determined that equipment was shared between this farm and the farm in the previous case.
[C]Antibody titers confirmed administration of the IBD vaccine.
[D]In a laboratory setting, 20% mortality was recorded in SPF birds with the same virus (11).
[E]It was later determined that personnel and equipment from this ranch were used to move birds during the time of infection at cases 1 and 2.
[F]Same owner as case 4.
[G]Same owner as cases 4 and 5.
[H]The owner has a restaurant and had previously purchased eggs from a retailer who was affiliated with the first two cases.
[I]Sequencing of the segment B region has not been successful.
[J]Affected birds were in Southern California.
[K]Birds over 2 wk were vaccinated against IBD.
[L]According to the ranch manager mortality was 5–10 times greater than normal.
[M]Segment B was not sequenced. However, this farm was the same farm as case 3 which had vvIBDV/serotype 2.
[N]Cases 13–23 represent four ranches in Northern California that were owned by the same owner as cases 3, 4, and 7. Two of these premises had other flocks that had been previously positive (cases 4 and 7). All the affected ranches were in the same region of Northern California.

northern California. Cases of vvIBD/standard, which geographically appeared to cluster in southern California, were all from a single flock and therefore lacked statistical power to demonstrate significant clustering. It is important to recognize that the segment B region was only sequenced if the segment A region was vvIBDV compatible. Therefore, we have no molecular or molecular epidemiological data with respect to other molecular types that represent endemic IBDV strains whether they are classic, standard, variant, or other unclassified or undetected strains.

One instance of statistically significant time-space clustering of vvIBDV/vvIBDV was observed (Table 3). All four of these ranches were owned by the same company. Therefore, it is likely there was some breakdown in biosecurity among the four farms. No significant temporal clustering was observed for any of the subtypes of vvIBDV. This is consistent with the seemingly random distribution of cases in the histogram presented in Fig. 1. In addition, the lack of temporal clustering may also be consistent with our negative IBDV surveillance results (data not shown) in peridomestic birds (wild birds geographically associated with affected premises) and other wild birds in California. If wild birds were infected with any of the three subtypes of IBDV noted in this study and if they were a significant source of transmission, significant temporal clustering would be expected. Consequently, at this point peridomestic and wild birds do not appear to play a significant role in the spread of the three subtypes of IBDV in California. However, it is important to recognize an epidemiological link between domestic chickens and wild birds (waterfowl) with respect to vvIBD has been identified elsewhere. Specifically, in South Korea in 2006–2007, wild bird isolates of the VP2 gene were closely related to vvIBDV isolates from domestic chickens in endemic areas (13). It is interesting to note that the prevalence of vvIBDV in the South Korean paper was 4.7% (5/107) and that 4/5 of the positive samples were from waterfowl (13). In California, we have tested the bursas of fewer than 50 wild birds including waterfowl and we have tested the allantoic fluid (not the preferred sample) from approximately 160 waterfowl (data not shown). Hence further testing is needed.

While there has been at least one documented case of vvIBDV transmission between wildlife and commercial flocks (13), the persistence of the virus even after cleaning and disinfection has been well established (6). Therefore, any sharing of equipment could play a direct role in transmission of vvIBDV between farms. This may have played a role in the initial spread of the virus between cases 1 and 2. However, the persistence of the virus in the environment coupled with how easily vvIBDV spread around the world between 1987 and 1992 (16) brings up the question of why vvIBDV/vvIBDV, vvIBDV/standard, and vvIBDV/serotype 2 have not spread further geographically. There are numerous possible explanations, including incomplete reporting and surveillance, minimal knowledge of symptoms of the disease on the part of poultry operation workers or BY chicken owners, or true low prevalence of the disease in California poultry flocks. The majority of the submissions from the Northern California area were submitted because there was

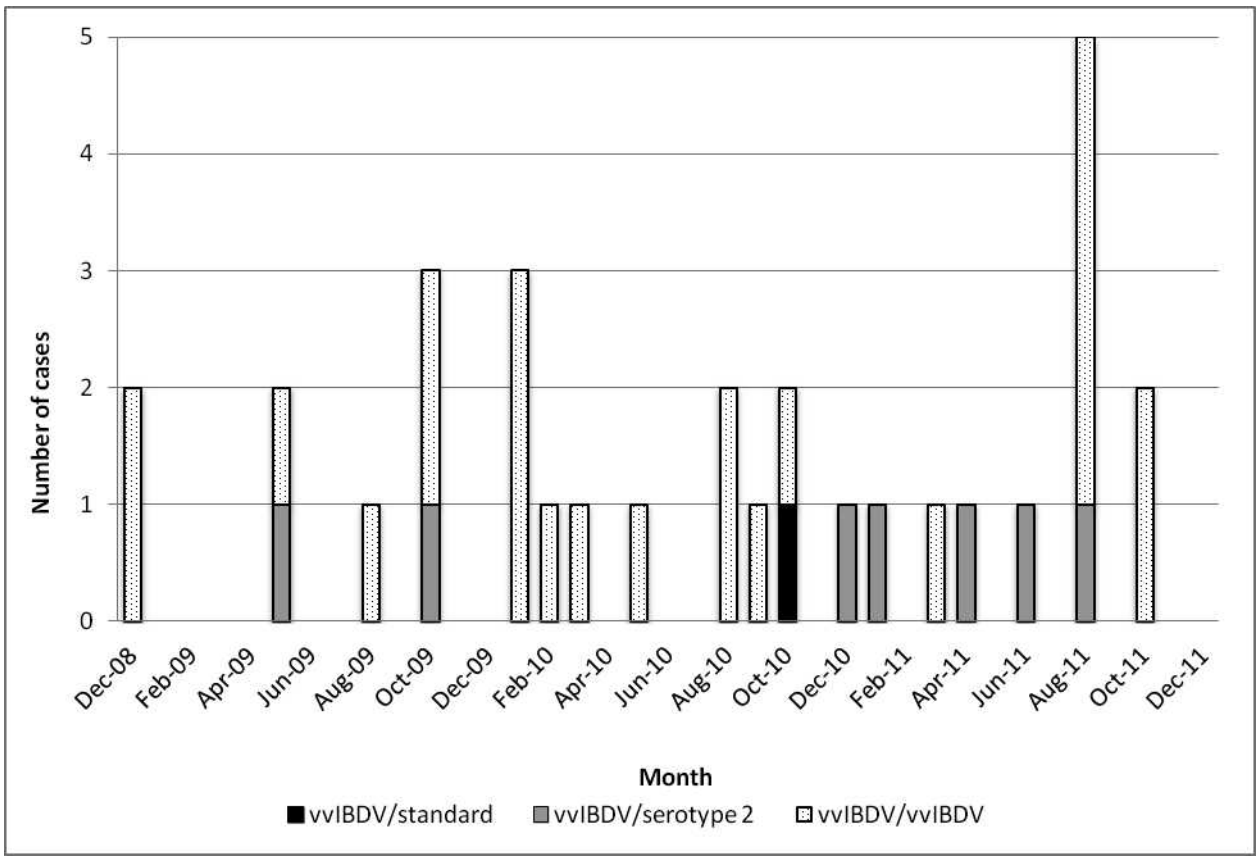

Fig. 1. Histogram of number of cases ($n = 31$), by subtype and month, of vvIBD diagnosed in California poultry flocks from December 2008 to December 2011. A case was defined as a premise that was affected with a vvIBD compatible virus once in the flock's life.

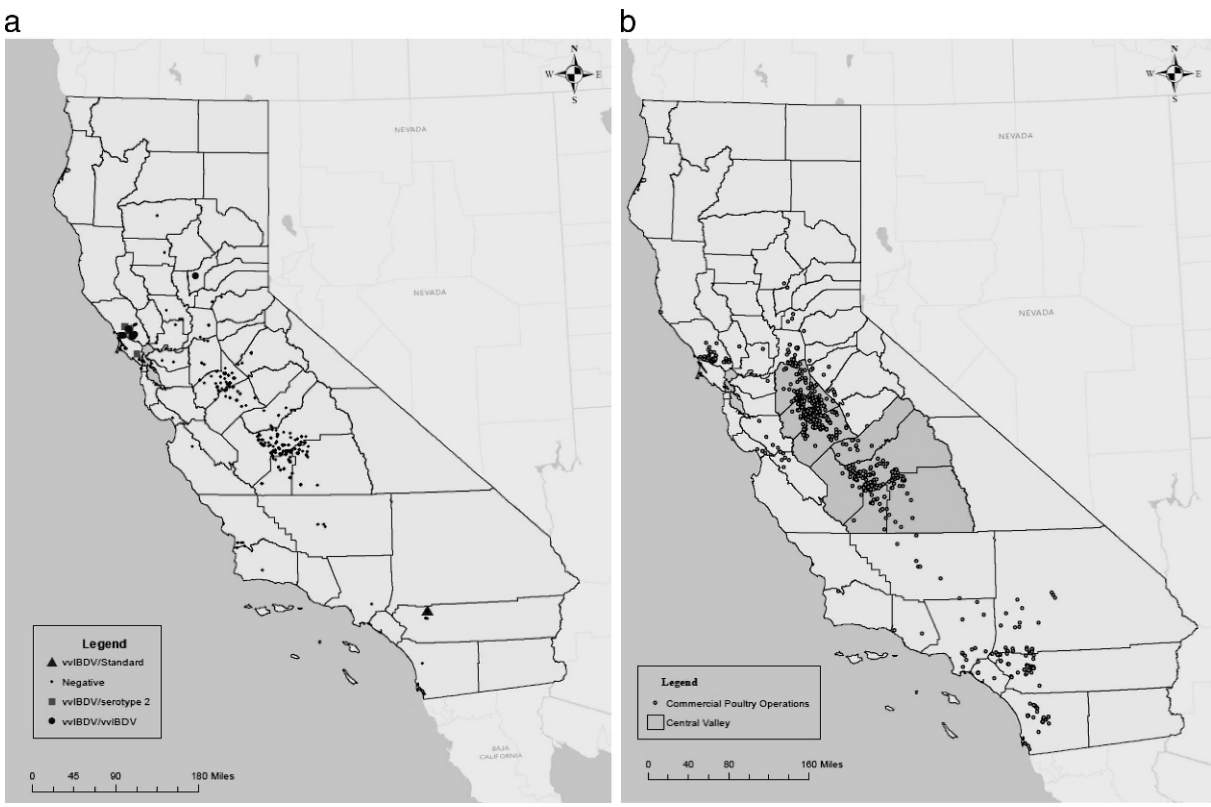

Fig. 2. (a) Location of PCR-tested poultry flocks relative to locations of California commercial poultry operations, 2008–2011. (b) Location of commercial poultry facilities in California. The Central Valley of California was defined as any county in the geographical central valley of California with over 20 commercial poultry premises. These counties included Fresno, Kings, Madera, Merced, San Joaquin, Stanislaus, and Tulare.

Table 2. Spatial cluster analysis of statistically significant non-overlapping spatial clusters of vvIBDV/vvIBDV, and vvIBDV/serotype 2 in California poultry flocks from 2008 to 2011.

Type of IBDV	Location	Radius (km)	Observed cases	Expected cases	Ratio (O/E)[A]	*P*
vvIBDV/vvIBDV	Northern California	30.6	22	2.07	10.63	≤0.001
vvIBDV/serotype 2	Northern California	35.8	7	0.64	11.0	≤0.001

[A]O/E = observed/expected.

something clinically abnormal with the chickens. In broilers, this type of submission procedure is likely to miss subclinical cases or flocks that have very mild symptoms as demonstrated by cases 4, 5, 7, and 13–23. Regardless, further surveillance and a greater understanding of the ecology of the virus will be essential toward measuring the true incidence and prevalence of these IBDV subtypes in California.

The State of California undertook a voluntary surveillance effort to try to determine the extent of the spread of vvIBDV. To date, the results of all testing done in the Central Valley have been negative. However, testing has only been completed in a small proportion (28%) of the commercial flocks in the Central Valley. Given that level of surveillance, the question of how likely it is that the vvIBD agent is not present in the Central Valley of California is important to address.

Using these parameters coupled with our current surveillance efforts the probability that the commercial poultry in the Central Valley is free of the vvIBD disease agent is between 28% and 81%, depending on the detection threshold (one or five infected farms) used. It is important to recognize that this confidence represents a best case scenario for the diagnostic testing and is therefore the maximum confidence that we can have based on the currently available surveillance data. Since all flocks on those farms were tested over a 3-yr period we cannot make the assumption that a single flock tested on a multiflock farm in 2008 or 2011 is still disease free (although birds were selectively submitted for diagnostics based on highest morbidity in order to enhance our surveillance efforts). In addition, the low probability of detection (28% or 81%) does not imply that affected flocks are present but provides minimal evidence that the commercial flocks in the Central Valley are free of vvIBD. It should be noted that our surveillance in the Central Valley was focused primarily on commercial poultry and not on BY flocks. Overall, the relative geographic isolation of the Central Valley from the affected vvIBDV/vvIBDV farms coupled with the knowledge and biosecurity precautions taken by the majority of these commercial producers should be considered relative to the knowledge that vvIBDV is highly contagious and persistent to the environment.

While the need to ''test more'' may seem obvious it is difficult to determine what the end-point would be; there are currently no OIE Terrestrial Code recommendations covering freedom from IBD or vvIBD (1). In addition, several other questions regarding the epidemiology, ecology, and evolution of the virus are still unresolved. Consequently, while the geographic clustering shows that distance from an affected facility appears to be a significant risk factor, the historical/epidemiological evidence presents several other trends worth noting. The mortality in the field of all three (vvIBDV/vvIBDV, vvIBDV/standard, and vvIBDV/serotype 2) subtypes of IBDV appears to be higher in layers (5%–30%) than in broilers (<1%). Voluntary vaccination appears to have benefits in mitigating the 5%–30% mortality rates that have been otherwise occurring in layer operations. With the low cost of vaccination, vaccinating flocks that are geographically clustered with previously positive facilities may be beneficial. Composting of litter material does not appear to inactivate the virus. The virus has re-appeared multiple times in the broiler facilities described in cases 4, 5, 7, and 13–23. Specifically, the farms windrow and compost the litter material in houses between flocks. Perhaps the most significant finding is the presence of the virus in BY bird populations (Table 1). Due to the lack of biosecurity coupled with a poor understanding of disease transmission, we believe that these vvIBDV subtypes will most likely continue to spread in potentially unexpected ways due to the nature of BY bird owners and how they transport and trade birds.

These risk factors and several others will be further evaluated statistically in a forthcoming epidemiological survey. Aside from investigating the described risk factors, a review of the literature presents some information with respect to IBDV, the variant forms of IBDV, and how they affect flock health. In general, vaccination and proper cleaning and disinfection are essential for attempting to control IBD (20). Effective vaccination depends on rapid and accurate diagnosis of the subtype present in a flock because vaccines based on the classic subtype of IBDV can fail to protect against challenges with a variant subtype (5). Consequently, continued molecular epidemiologic surveillance is essential for responding appropriately.

In summary, between 2008 and 2011 at least three different subtypes (vvIBDV/vvIBDV, vvIBDV/standard, and vvIBDV/serotype 2) of IBDV were noted in California poultry based upon sequencing results. Results of the clustering tests demonstrate that statistically significant spatial clustering of two subtypes of vvIBDV—vvIBDV/vvIBDV and vvIBDV/serotype 2—occurred in California during the study period, 2008 to 2011. One instance of statistically significant time-space clustering of vvIBDV/vvIBDV was observed. No temporal clustering was noted. While currently in the United States, vvIBDV and its subtypes may only be a ''California problem,'' it is important to recognize the potential for vvIBDV to spread across the continent. Maintaining an effective statewide surveillance program is essential toward containment of the disease from the rest of the United States. Consequently, a vigilant epidemiological surveillance program where poultry farmers (commercial and BY) regularly submit suspect birds for vvIBD testing could greatly enhance predictions of the actual disease prevalence, particularly if the operations are located within the identified cluster radii (Tables 2 and 3) around a positive case.

Table 3. Time-space cluster analysis of statistically significant time-space clusters of vvIBDV/vvIBDV, in California poultry flocks from 2008 to 2011. Time-space clusters over 1 yr or over 500 km were excluded.

Time	Location of cluster center	Type of IBDV	Radius (km)	Observed cases	Expected cases	Ratio (O/E)	*P* value
Aug. 14, 2011 to Nov. 5, 2011	Northern California	vvIBDV/vvIBDV	1.64	4	0.70	5.75	0.012

REFERENCES

1. Animal Health Australia. Disease strategy: infectious bursal disease caused by very virulent IBD virus or exotic antigenic variant strains of IBD virus (Version 3.0), Australian Veterinary Emergency Plan (AUSVETPLAN), Edition 3. Primary Industries Ministerial Council, Canberra, ACT. pp. 1–44. 2009.

2. Ashraf, S., G. Abdel-Alim, and Y. M. Saif. Detection of antibodies against serotypes 1 and 2 infectious bursal disease virus by commercial ELISA kits. Avian Dis. 50:104–109. 2006.

3. Brooker, S., S. Clarke, J. K. Njagi, S. Polack, B. Mugo, B. Estambale, E. Muchiri, P. Magnussen, and J. Cox. Spatial clustering of malaria and associated risk factors during an epidemic in a highland area of western Kenya. Trop. Med. Int. Health 9:757–766. 2004.

4. Cannon, R. M., and R. T. Roe. Livestock disease surveys. A field manual for veterinarians. Department of Primary Industry, Australian Government Publishing Service, Canberra, ACT. 1982.

5. Ching, W. C., P. Rubinelli, and L. Tsang. Molecular detection and differentiation of infectious bursal disease virus. Avian Dis. 51:512–526. 2007.

6. Eterradossi, N., and Y. M. Saif. Infectious bursal disease. In: Diseases of poultry, 11th ed. Iowa State Press, Ames, IA. pp. 185–208. 2008.

7. Gao, H. L., X. M. Wang, G. Y. L. Gao, and C. Y. Fu. Direct evidence of reassortment and mutant spectrum analysis of a very virulent infectious bursal disease virus. Avian Dis. 51:893–899. 2007.

8. Jackwood, D. L., B. M. Crossley, S. T. Stoute, S. S. Wagner, P. R. Woolcock, and B. R. Charlton. Diversity of genome segment B from infectious bursal disease viruses in the United States. Avian Dis. 56:165–172. 2012.

9. Jackwood, D. J., and S. E. Sommer-Wagner. Molecular epidemiology of infectious bursal disease viruses: distribution and genetic analysis of newly emerging viruses in the United States. Avian Dis. 49:220–226. 2005.

10. Jackwood, D., and S. Sommer-Wagner. Genetic characteristics of infectious bursal disease virus from four continents. Virology 365:369–375. 2007.

11. Jackwood, D. J., S. E. Sommer-Wagner, B. M. Corssley, S. T. Stoute, P. R. Woolcock, and B. R. Charlton. Identification of pathogenicity of a natural reassortment between a very virulent serotype 1 infectious bursal disease virus (IBDV) and a serotype 2 IBDV. Virology 420:98–105. 2011.

12. Jackwood, D. J., B. Sreedevi, L. J. LeFever, and S. E. Sommer-Wagner. Studies on naturally occurring infectious bursal disease viruses suggest that a single amino acid substitution at position 253 in VP2 increases pathogenicity. Virology 377:110–116. 2008.

13. Jeon, W.-J., E.-K. Lee, S.-J. Joh, J.-H. Kwon, C.-B. Yang, Y.-S. Yoon, and K.-S. Choi. Very virulent infectious bursal disease virus isolated from wild birds in Korea: epidemiological implications. Virus Res. 137:153–156. 2008.

14. Kulldorff, M. A spatial scan statistic. Commun. Stat. Theory Meth. 26:1481–1496. 1997.

15. Kulldorff, M., R. Heffernan, J. Hartman, R. Assuncao, and F. Mostashari. Space-time permutation scan statistic for disease outbreak detection. PLoS Med. 2:216–224. 2005.

16. [O.I.E.] World Organisation for Animal Health. 2011. Article 1.4.2. In: Terrestrial Animal Health Code. [Internet]. [accessed 2012 April 15]. Available from: http://www.oie.int/doc/ged/010905.pdf.

17. Rauf, A., M. Kharti, M. V. Murgia, K. Jung, and Y. M. Saif. Differential modulation of cytokine, chemokine and Toll-like receptor expression in chickens infected with classical and variant infectious bursal disease virus. Vet. Res. 42:85. 2011.

18. Saif, Y. M., and N. Eterradossi. Infectious bursal disease. In: Diseases of poultry, 12th ed. Y. M. Saif, ed. Blackwell, Ames, IA. pp. 185–209.

19. Stoute, S. T., D. J. Jackwood, S. E. Sommer-Wagner, G. L. Cooper, M. L. Anderson, P. R. Woolcock, A. A. Bickford, C. G. Senties-Cue, and B. Charlton. The diagnosis of very virulent infectious bursal disease in California pullets. Avian Dis. 53:321–326. 2009.

20. Vaziry, A., D. Venne, D. Frenette, S. Gingras, and A. Silim. Prediction of optimal vaccination timing for infectious bursal disease based on chicken weight. Avian Dis. 51:918–923. 2007.

ACKNOWLEDGMENT

We would like to thank Mackenzie Johnson for her help editing this manuscript.