

Epidemiologic Analyses of Virulent Newcastle Disease in Poultry in California March 2021



Suggested bibliographic citation for this report:

USDA-APHIS (2021). "Epidemiologic Analyses of Virulent Newcastle Disease in Poultry in California, March 2021." USDA:APHIS:VS:Center for Epidemiology and Animal Health. Fort Collins, CO. March 2021. 101 pgs.

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Contents

Executive Summary	2
Introduction	5
A. Disease Overview.....	5
B. Description of Outbreak.....	6
C. Surveillance Overview	11
D. References.....	13
II. Phylogenetic Analysis and Diagnostics	14
A. Virulent Newcastle Disease Virus	14
Methods	14
Results	14
B. Comparison to Other Viruses/Lineages	19
C. Diagnostics	20
D. References.....	20
III. Population at Risk.....	21
A. Predicting Areas of Backyard Bird Ownership	21
Methods	21
Results	21
Summary.....	24
IV. Epidemiologic Risk.....	25
A. Case Control Study	25
Methods	25
Results	25
Summary.....	27
Summary of Historical Epidemiologic Risk Factors.....	27
B. Epidemiologic Investigation of vNDV-Infected Commercial and Backyard Non-Commercial Laying Chicken Premises	29
Methods	29
Results	30
C. References.....	36
V. Estimating Disease Spread.....	37
A. Flock Disease Spread Model—Early Outbreak.....	37
Methods	37
Results	37
B. Comparing Alternative Control Strategies—Mid-Outbreak.....	39
Methods	39
Results	40
C. Estimating Within-Flock Transmission Parameters and Predicting the Time to Detect vND in Unvaccinated Flocks	41
Methods	41
Results	42
D. Predicted Disease Mortality and Infection Prevalence in vNDV Infected Flocks Using a Disease Transmission Simulation Model	42
Methods	43
Results	43

- Conclusions..... 48
- E. Estimating the Time of Disease Introduction in vNDv Infected Commercial Layer Barns Using Egg
 - Production and Mortality Data 49
 - Summary..... 49
 - Methods 49
 - Results 50
 - Discussion 53
- F. Spatial and Spatiotemporal Patterns of the Outbreak 55
 - Methods 55
 - Results 55
 - Conclusions..... 60
- G. Measuring the Spatial Dependence of Virulent Newcastle Disease Transmission Risk 60
 - Methods 61
 - Results 62
 - Conclusions..... 66
 - Implementation During Outbreak 66
- H. References..... 67
- VI. Acknowledgements 69**
- Appendix A: Questionnaire 70**
- Appendix B: Modeling Scenario Design 75**
- Appendix C. Predicted Disease Mortality and Infection Prevalence Under Additional Flock Size And Vaccination Scenarios..... 77**
 - Predicted mortality and disease prevalence in a 50-bird unvaccinated backyard flock 79
 - Predicted mortality and disease prevalence in a 50-bird vaccinated backyard flock..... 81
 - Predicted mortality and disease prevalence in a 5000-bird unvaccinated commercial flock 82
 - Predicted mortality and disease prevalence in a 20,000-bird unvaccinated commercial flock 84
 - Predicted mortality and disease prevalence in a 5000-bird vaccinated commercial flock 86
- Appendix D. Technical Details for estimating the time of vND introduction in Commercial Vaccinated Layer Flocks..... 88**
 - A. Overview 88
 - B. Estimating prior distributions 88
 - C. Goodness of Fit measure calculation 89
 - D. Model Implementation and coding validation 91
 - Results 92

List of Figures and Tables

Figure 1. Counties with confirmed findings of vND from 17 May 2018 to 4 June 2019.	10
Figure 2. California vND weekly case detection curve based upon the date the case definition was met for a presumptive positive flock, by day from 17 May 2018 to 25 February 2020.....	11
Figure 3. Distribution of analyzed viruses by virus group and date of sample collection through November 2019.	15
Figure 4. Distribution of the RV-a vNDV subgroup in California from 19 December 2018 to 11 May 2019..	16
Figure 5. Distribution of subgroup of vNDV-02 viruses which occurred after August 1, 2019..	18
Figure 6. Phylogenetic analysis based on the full-length nucleotide sequence of the fusion gene of isolates representing Newcastle disease virus class II, genotype V	19
Figure 7. Comparison of predicted number of backyard poultry flocks (boxplots) with observed distribution of backyard poultry flocks (red).	22
Figure 8. Predictive variables used in the generalized additive model.	23
Figure 9. Smoothing splines describing the relationship between number of poultry flocks and non-linear predictor variables.	23
Figure 10. Predicted geographic distribution of backyard poultry flocks in southern California. The map presents the density of flocks per km ²	24
Figure 11. Predicted cumulative mortality percent and prevalence of infectious birds on various days post exposure in a 20000 vaccinated commercial cage free layer flock using baseline vND spread parameters.	44
Figure 12. Predicted daily mortality on various days post exposure in a 20 000-bird vaccinated commercial cage free layer flock using baseline vND spread parameters	44
Figure 13. Predicted cumulative mortality percent and prevalence of infectious birds on various days post exposure in a 400-bird unvaccinated backyard flock using baseline vND spread parameters.	45
Figure 14. Predicted daily mortality on various days post exposure in a 400 bird unvaccinated backyard flock using baseline vND spread parameters.....	46
Figure 15. Predicted cumulative mortality percent and prevalence of infectious birds on various days post vND exposure in a 400-bird vaccinated backyard flock.	47
Figure 16. Predicted daily mortality on various days post vND exposure in a 400-bird vaccinated backyard flock.....	48
Figure 17. Model-fitted egg production rate curves from the approximate Bayesian computation and observed egg production rate for Barn A.	51
Figure 18. Model fitted disease plus normal mortality from the approximate Bayesian computation and the observed daily mortality for Barn A.	52
Figure 19. Posterior distribution for the adequate contact rate for Barn A.	53
Figure 20. Histogram of the Posterior distribution for the time of vND introduction for Barn A.	53
Figure 21. Cumulative number of confirmed and presumptive positive vND premises detected in California from 16 May 2018 to 22 February 2020.	56
Figure 22. Location of spatial (upper) and spatiotemporal (lower) clusters of vNDV-01 premises and the relative risk of cases occurring within census blocks in California from 16 May to 22 February 2020.	57
Figure 23. Location of spatial (upper) and spatiotemporal (lower) clusters of vNDV-02 premises and the relative risk of cases occurring within census blocks in California from 16 May to 22 February 2020.	58
Figure 24. Time period of occurrence of spatiotemporal clusters of vND-01 and vND-02 in California from 16 May 2018 to 22 February 2020.....	60

Figure 25. Spatial dependence of vND infected premises and the percent of infected premises located within the specific distance and occurring within 21 days of each other.	64
Figure 26. Spatial dependence of vND infected premises located within the specified distance and occurring within 21 and 120 days of each other..	65
Table 1. Number of vND confirmed and presumptive positive premises, by county and dates of earliest confirmation in each county through 25 February 2020.	9
Table 2. The number of distinct sampled infected commercial and independent premises, laboratory accessions, and samples collected from infected premises in the RQA as part of vNDV surveillance by production type.	12
Table 3. The number of distinct sampled uninfected commercial and independent premises, laboratory accessions, and samples collected from negative premises in the RCA as part of vNDV surveillance by production type.	13
Table 4. Detection timeline for the RV-a vNDV subgroup in California between 19 December 2018 to 11 May 2019 by sample collection date.	17
Table 5. Characteristics of backyard case premises (confirmed/presumptive positive for vND), dangerous contact (DC) premises, control premises (C), and odds ratios (OR) and p-values calculated by univariate logistic regression (dangerous contacts excluded).	28
Table 6. Adjusted odds ratios (OR) for significant risk factors identified in multivariate regression analyses.	28
Table 7. Production types, counties, confirmation dates, and numbers of euthanized birds on ten vNDv infected commercial and backyard/non-commercial layer chicken premises.	32
Table 8. Numbers of birds, numbers of flocks, numbers of houses in use and numbers of employees reported by vND infected case premises (n=10) and control premises (n=28) located in the three-county regional quarantine area of southern California.	33
Table 9. Management characteristics of vND infected case premises (n=10) and control premises (n=28) in the three-county regional control area of southern California.....	34
Table 10. Qualitative summary comparison of the four alternative response options examined.	40
Table 11. Predicted days post onset of infectiousness to attain various cumulative mortality levels in a 20,000-bird vaccinated commercial cage free layer flock using baseline vND spread parameters.	45
Table 12. Predicted days post onset of infectiousness to reach various cumulative mortality levels in a 400-bird unvaccinated backyard flock using baseline vND spread parameters.	46
Table 13. Predicted days post onset of infectiousness to reach various cumulative mortality levels in a 400-bird vaccinated backyard flock using baseline vND spread parameters.	48
Table 14. Estimated time of introduction and adequate contact rate for each of the four barns.	52
Table 15. Spatiotemporal clusters of vND cases in California from 16 May 16 2018 to 22 February 2020. ^a	59

EXECUTIVE SUMMARY

In May 2018, an owner brought several ill exhibition chickens to a veterinary clinic in southern California. The birds were displaying signs of virulent Newcastle disease (vND). Biological samples were collected from the chickens and sent the California Animal Health and Food Safety (CAHFS) Laboratory where vND virus was detected. The U.S. Department of Agriculture's (USDA) National Veterinary Services Laboratories (NVSL) confirmed vND in these birds on 17 May 2018. Between 17 May 2018 and 31 May 2020, 476 premises were confirmed as infected with the virus. The last infected backyard non-commercial chickens were confirmed on 29 November 2019 and the last infected exhibition establishment was confirmed on 25 February 2020.

Once initial response efforts were in place, the USDA Animal and Plant Health Inspection Service's (APHIS) Veterinary Services initiated a series of epidemiologic investigations and studies, which were undertaken collaboratively with bird owners, State agriculture personnel, and the USDA's Agricultural Research Service (ARS). This report provides a summary of the epidemiologic analyses conducted in support of this outbreak and is intended to provide a better understanding of how the vND virus was introduced and transmitted.

The outbreak predominantly affected exhibition chickens in an area crossing three Southern California counties: San Bernardino, Riverside, and Los Angeles. Additional isolated cases were detected in Ventura County, CA, Alameda County, CA, San Diego, CA, Utah County, UT, and Coconino County, AZ. Genetic analysis supports a single introduction followed by secondary spread. Lack of epidemiologic data regarding the index premises, and temporal and geographical gaps in available genetic data, contribute to the uncertainty surrounding the origin of the outbreak. Following introduction into CA, divergence of the virus into two sub-groups appeared early on and, where epidemiologic data is available, was useful to gain insights on virus spread. Although geospatial clustering of virus sub-groups was observed, the presence of different virus sub-groups in each of the major affected areas indicated virus movement within, and between, affected areas.

The affected counties in CA have a high density of backyard or exhibition flocks, but such flocks are not typically registered, and their exact locations were unknown. Using a Bayesian hierarchical model, previously identified socioeconomic and demographic variables found to be associated with urban poultry ownership were used to estimate the probability of backyard flocks in this area. Results suggested that a single 10-km zone could have as many as 4,000 backyard flocks, and that the greater Los Angeles area might have more than 11,000. Modeled backyard ownership maps helped to inform surveillance and response efforts.

Analyses of surveys conducted at case and control premises (including dangerous contacts)¹ identified flock size, ownership of exhibition birds, high proportions of roosters in flocks, and the use of housing that allows contact with wild birds as risk factors for vND in this population. The percentage of premises reporting the use of Newcastle vaccine was low overall; however, its use was higher in case premises. Improper vaccination of backyard birds is a concern due to improper administration leading to development of reservoirs of vND. An epidemiologic investigation into 10 vND infected commercial and

¹ Dangerous contact premises are defined as premises with backyard/exhibition birds that are high risk due to either an epidemiologic link or proximity to infected premises.

backyard non-commercial laying chicken premises and 28 control premises found that some factors and management practices were shared across infected farms; however, the significance of these similarities was difficult to interpret given the small number of infected commercial and non-commercial laying chicken farms and the study design. All cases and controls reported vaccination of their flocks to protect against Newcastle disease.

Initial results from disease-spread and control simulations suggested that local disease spread would become increasingly important as the outbreak increased in size. This type of disease spread is distance-dependent and represents mechanisms of spread that are difficult to trace, such as movement of free ranging birds, wildlife, or fence-line contact. Good biosecurity practices and measures are the best way to reduce local spread, but completely preventing this type of spread is difficult. As the outbreak progressed, modeling scenarios were developed to compare alternative control options and resource levels for response. These scenarios found that minimal response levels (including low surveillance and depopulation capacities) were unlikely to prevent continued disease spread in backyard flocks. Rapid and targeted surveillance, depopulation, and disposal were most effective at minimizing outbreak size and severity. The largest and longest simulated outbreaks frequently involve significant disease spread within Los Angeles County, irrespective of the selected response option.

Using experimental data available from peer-reviewed literature and unpublished data provided by the USDA, ARS, Southeast Poultry Research Laboratory (SEPRL), analysts estimated the mean latent period for this virus was 0.40 days, and the mean infectious period was 4.33 days in unvaccinated birds. Using these values, analysts estimated the time to detect vND in an unvaccinated, 50-bird backyard flock based on observation of increased mortality (two or more dead birds within a 3-day period) to be from 4 to 7 days. Building on this work, a stochastic within-flock vND transmission model was developed to predict the prevalence of infectious birds and cumulative mortality over time for both vaccinated and unvaccinated flocks. In large vaccinated flocks, it may take 14 to 22 days after the onset of infectiousness for the cumulative mortality to reach 2 percent of the starting flock size. In contrast, in an unvaccinated backyard flock, a 50 percent cumulative mortality may be seen within a week. This information was used to help guide on-farm surveillance and monitoring efforts.

The identification of significant spatial and spatiotemporal clustering patterns of vND in California from May 2018 to February 2020 supported control strategies of targeting high risk areas for disease spread with enhanced surveillance and depopulation activities. The primary spatial and spatiotemporal clusters were located within Riverside and San Bernardino Counties, identifying these areas as the location of the highest occurrence of vND cases detected from 16 May 2018 to 22 February 2020. These clusters occurred during the first seven months of the outbreak, corresponding to the increase in infected premises that were detected during this time as well as further disease spread over the next year within the Regional Quarantine Area (RQA)². Examination of the spatial dependence of vND transmission risk from 342 cases from the start of the outbreak through 1 May 2019, found evidence for both local and long-distance spread of the virus. Most infected premises (75.6 percent) were found to be within 250 m of another infected premises, with over 95 percent of infected premises occurring within 1.5 km of another infected premises. However, the increased probability of premises being infected at longer

²RQA was implemented on 27 February 2019 by the California Department of Food and Agriculture and encompassed all of Los Angeles County and most of Riverside and San Bernardino Counties.

distances, from 2.5 to 6.0 km depending on virus sub-group, highlight the risk of spread by movement of infected poultry or fomites out of affected areas. Statistically significant spatial clustering over longer time periods (42 to 120 days apart in confirmation date) was also observed at distances between 5.0 and 8.0 km. These results indicate longer-term disease transmission, which may occur due to undetected, infected premises or violations in the fallow period. The results of this analysis were used to guide surveillance and depopulations efforts for backyard/exhibition premises between July 2019 and the end of the outbreak.

On June 1, 2020, USDA APHIS certified that the United States of America (U.S.A.) satisfied the World Organisation for Animal Health (OIE) criteria for eradication of virulent Newcastle disease from poultry, as defined by the OIE, and exhibition birds. Subsequently, CDFA announced an end to the vND quarantine in Southern California through release of the RQA, an area where millions of commercial and backyard poultry live. This action once again allowed poultry to move freely within California without a CDFA permit. All necessary actions and surveillance requirements were completed in accordance with the OIE Terrestrial Animal Health Code with no further detections of the disease. The USDA APHIS now considers the vND event officially closed and resolved. The total cost of indemnity approved by USDA during this outbreak was \$6.7 million (\$3.7 million to affected commercial producers and \$3 million to backyard flock owners).

To guard against the risk of subsequent outbreaks, CDFA and USDA in partnership with many bird enthusiasts in Southern California are committed to on-going monitoring for disease and continual support for biosecurity training. All backyard poultry and exhibition bird owners and commercial operations are encouraged to practice biosecurity measures to help prevent the introduction of disease when people enter or depart the premises, to routinely check birds for signs of illness, and to report any incidence of suspected vND or other bird diseases.

INTRODUCTION

California and USDA-APHIS initiated epidemiologic and genetic investigations in response to the vND outbreak in backyard/exhibition chickens in Southern California. These investigations provided a better understanding of factors associated with vND virus transmission among backyard chickens³ and other susceptible species. These investigations included:

- Analysis of the phylogenetic characteristics of the virus,
- Estimation of the probability of homes in Southern California owning backyard birds,
- A field-based study of backyard case and control premises using data collected through site visits and interviews with backyard-chicken owners,
- A field-based study of commercial poultry case premises using data collected through site visits and interviews,
- An epidemiologic disease-spread simulation model of vND spread among bird-owning households in Southern California and comparison of alternative control options,
- An examination of within-flock disease transmission and the impact on the time to detection in small unvaccinated flocks,
- Prediction of disease mortality and infection prevalence in vND infected flocks of varying flock sizes and vaccination status,
- Estimation of the time of disease introduction in vND-infected commercial layer barns using egg production and mortality data,
- An analysis of spatial and spatiotemporal patterns of disease and the spatial dependence of vND transmission risk.

This report is a supplement to previous epidemiologic investigations of this outbreak reported previously. This report includes the results from these investigations, to provide producers, industry, and other stakeholders with epidemiologic information and to archive the analytical work performed to support future outbreak response.

A. Disease Overview

Newcastle disease is the cause of regular, frequent poultry epizootics throughout Africa, Asia, Central America, and parts of South America. The disease is caused by strains of avian paramyxovirus-1, also known as Newcastle disease virus, which can be classified into three pathotypes based on their virulence in chickens. The World Organisation for Animal Health (OIE) defines Newcastle disease as infection caused by highly virulent strains of APMV-1 viruses. This virulent form of Newcastle disease (vND) is considered a foreign animal disease in the United States.

³ For the purposes of this report, the term backyard flock may also refer to birds raised for exhibition.

Clinical signs of vND vary and can include respiratory, neurological, reproductive, and intestinal signs. During this outbreak, clinical signs seen in chickens include loss of appetite, difficulty breathing, nasal discharge/ocular discharge, swelling around the eyes, diarrhea, blue combs, and death. Morbidity of unvaccinated chickens infected with vND virus strains can reach 100 percent, and mortality ranges from 70 to 100 percent. The severity of disease produced varies with the host species and the strain of the virus. Many other avian diseases present with clinical signs similar to vND; therefore, laboratory testing is necessary to distinguish between diseases.

Newcastle disease is transmitted by inhalation or ingestion, and birds shed the virus in both feces and respiratory secretions. The virus can infect many species of domestic and wild birds. Chickens are highly susceptible, and other gallinaceous birds such as turkey, quail, and guinea are also susceptible. There are two species-adapted viruses that are genetically distinguishable from those found in poultry in the absence of direct transmission: one is maintained in pigeons and doves, and another in double-crested cormorants (Brown and Bevins, 2017). Parrots have been reported to be infected with virulent viruses and have the potential to shed virus for long periods without showing clinical signs; however, data supporting virus maintenance in these species is lacking.

Vaccination of commercial poultry against Newcastle disease is common in the Americas, including the United States. The classical vaccine strains are distinguishable from other viruses by genome sequencing. Widespread vaccination of poultry was implemented in Mexico and several Central American countries in the early 2000s, and since this time divergence of subgenotypes circulating in vaccinated poultry has been documented (Susta et al., 2014; Garcia et al., 2013). Outbreaks of vND occurred in California, Nevada, and Arizona in 2002-2003 and in Texas in 2003.

B. Description of Outbreak

16 May 2018 to 4 June 2020

On 16 May 2018, the California Department of Food and Agriculture (CDFA) reported vND in sick exhibition chickens presented to a veterinary clinic in Los Angeles County (Figure 1). Biological samples were collected from the chickens and sent the California Animal Health and Food Safety (CAHFS) Laboratory where vND virus was detected. The National Veterinary Services Laboratories (NVSL) confirmed vND virus (vNDV) on 17 May 2018. This confirmation represented the first case of vND, (formerly referred to as exotic Newcastle disease) in the United States since 2003. Officials were first alerted to the possibility of a new finding of vND when an owner presented sick chickens to a California veterinary clinic. The CDFA responded to the incident by creating 3-km control areas around the premises associated with the index case and began targeted surveillance and outreach, including feed stores and known exhibition bird premises.

On 24 May 2018 NVSL confirmed vNDV in an exhibition premises in San Bernardino County. On 26 May 2018, a USDA-APHIS incident management team joined the unified incident command in California. By this time, five premises had been confirmed in San Bernardino County and two in Los Angeles County. On 30 June 2018, a premises in Riverside County was confirmed. On 14 August 2018, vNDV was confirmed in Ventura County.

On 25 September 2018, NVSL confirmed vNDV in a live bird market in Los Angeles County. The owner of the vND-infected live bird market in Los Angeles County reported first observing clinical signs

approximately two weeks prior to presumptive diagnosis. Over the four weeks prior to reporting disease, the market received 43 shipments of live birds from four suppliers: 37 shipments of broilers, 4 shipments of spent hens, and 2 shipments of ducks. Bird shipment sizes ranged from 15 to 558 birds (mean=181 birds per shipment). Suppliers used dedicated cages that were washed and sanitized between shipments to transport birds. Suppliers typically made stops at more than one live-bird market on their routes. The market was visited by one renderer, typically three times per week. The owner of the market reported rarely receiving birds from the community, and no community birds were received in the 60 days prior to the onset of clinical signs. Active surveillance of other live bird markets resulted in an additional confirmed detection in a live bird market in March 2019.

On 14 December 2018, NVSL confirmed vNDV in a chicken pullet ranch in Riverside County representing the first detection in a commercial poultry operation. Three additional commercial premises in Riverside County (table egg layer facilities) were confirmed for vNDV in January and February 2019. On 24 January 2019, a backyard non-commercial laying hen operation was confirmed as positive in San Bernardino County. Four additional backyard non-commercial laying hen operations were confirmed between February and March 2019. Of the 10 infected commercial and backyard non-commercial premises, six were not reported to have had any clinical signs and were detected on routine mandatory surveillance, while the other four were reported to have clinical signs that included mild decreased egg production or increased mortality.

On 17 January 2019, vNDV was confirmed in Utah County, Utah, the first confirmed infection outside of southern California. Additional confirmations outside of southern California occurred in Alameda County, CA on 13 March 2019 and Coconino County, AZ on 1 April 2019. From 17 May 2018 to 25 February 2020, 496 confirmed or presumptive positive premises were identified in six California counties, one Utah county and one Arizona county (Figure 2, Table 1).

An infected exhibition flock premises with multiple bird owners was detected in Utah County, Utah in January 2019. A concerned individual contacted the Utah State Veterinarian's office to report sick and dying birds with clinical signs consistent with vND. The owners were contacted, and samples submitted for testing on 15 January 2019. The owners reported bringing birds from California in early January. NVSL confirmed vNDV in the flock on 17 January 2019. All but one of the owners depopulated their flocks themselves, while state/federal officials depopulated the remaining owner's birds. Depopulation of the flock was completed on 19 January 2019. Construction vehicles, equipment, storage units, cages and pens were cleaned and disinfected, and the premises placed under extended fallow quarantine on 20 January 2019. All commercial layer operations in the area were notified of the suspect case and advised to heighten biosecurity. Surveillance yielded no further positive cases.

A single infected pet chicken premises was identified in Flagstaff, Coconino County, AZ. The owner reported lethargy, upper respiratory signs and death in numerous birds starting on 22 March 2019. Officials were first alerted to the possibility of vND on 27 March 2019 when the owner's veterinarian reported that pathologic findings consistent with vND had been found on necropsy of one of the chickens. Oropharyngeal samples from remaining chickens on the premises were confirmed to be positive for vND on 1 April 2019. All remaining birds were depopulated, and the premises placed under extended fallow quarantine on 2 April 2019. Census, outreach, and surveillance testing of premises with poultry within 1 km of the index case was completed on 9 April 2019. All surveillance samples collected for premises with poultry within the 1 km zone and additional premises on border of 1 km zone (n=69)

tested negative by vNDV PCR at Arizona Veterinary Diagnostic laboratory. Samples from chicks at the local feed store where the owners' chicks were sourced were found to be negative for vNDV. Although no epidemiologic links were identified, the virus genetic data connects this detection to other infected premises in California. No further cases in AZ were detected.

On 27 February 2019, the California State Veterinarian modified Southern California's quarantine area to further restrict bird movement. The quarantine mandated the reporting of sick birds and prohibited poultry owners from moving birds in all of Los Angeles County, and in large areas of San Bernardino and Riverside counties. The quarantine required bird owners to allow diagnostic testing, to isolate poultry from other species, to cease exhibitions, to stop the shipping and receiving of birds, and to enhance biosecurity.

On 7 March 2019, the United State Postal Service issued an industry alert indicating that they would no longer allow shipments of poultry into or out of zip codes 90000-93599, which affected the San Diego, Los Angeles, Santa Ana and Sierra Coastal districts. The quarantine was updated on 21 March 2019 to include all birds as well as hatching and embryonated eggs. Reminders of the ban were also issued on 8 November 2019 and 13 January 2020. This quarantine was officially lifted on 1 June 2020.

Beginning in June 2019, intensive control activities combined with diagnostic testing strategies allowed for the release of control areas to begin. Active work to identify and conduct outreach to all retail feed stores and their customers was on-going throughout the outbreak. On 14 August 2019 a retail feed store, previously located within a control area and identified through feed store outreach, had poultry which were confirmed positive for vND. Epidemiologic investigation revealed that the store was a location where unwanted poultry were left, often without any indication of their origin. Although tracing from the infected premises was challenging, the presence of positive birds was a valuable indicator of on-going viral circulation in the area. A new control area was established, and surveillance conducted in the surrounding area.

On 24 August 2019, an antibody production facility in San Diego County, which used chickens to produce eggs for the antibody production process, noted clinical signs consistent with vND, including diarrhea, depression, sudden death, discharge around eyes, coughing/gasping, paralysis, and swelling around the eyes and neck. The premises was confirmed infected with vND on 31 August 2019. The chickens at the facility were kept indoors, caged and in pens. Although other companies operated at the same location, no other companies housed any birds at this location. Trace-out investigations subsequently identified a vND positive small exhibition flock. Poultry on the premises were housed outdoors in adjacent wire enclosures and included chickens, ducks, pigeons, and doves. This cluster of detections starting 14 August 2019 all fell into the same vNDV-02 subgroup and did not represent a new introduction of the virus, based upon molecular studies and epidemiologic investigation.

Release testing for all control areas was initially completed on 16 October 2019, and all control areas were closed. However, freedom from disease testing was suspended on 15 November 2019, after diagnostic testing of an exhibition chicken flock in San Bernardino County with high mortality and clinical signs compatible with vND. The flock was confirmed positive on 16 November 2019 and a new control area established. Additional surveillance and outreach activities resulted in an additional 26 detections, primarily in small exhibition chicken flocks on the border between San Bernardino and Riverside Counties. These detections were all epidemiologically linked, and investigations identified several risk factors for disease spread in this area. Many of the infected premises frequented a feed store that had a

drive-through arrangement for selling feed in bulk. November is also the beginning of many exhibition activities, which while prohibited, were likely driven underground, resulting in illegal bird movements in the area. Twenty-three of the 27 cases detected in this cluster were in exhibition chickens. An additional detection of a pet chicken located in a retail pet store in Los Angeles County was also found to be linked to the San Bernardino cases, where the owner of the store lived and likely carried the virus from the area to the pet store. A total of three control areas were established in Los Angeles, Riverside, and San Bernardino counties. The last detection in California occurred on 25 February 2020. The last control area was closed on 23 March 2020 after testing of all premises in the control area was completed with negative test results.

Freedom from disease testing was completed, with ongoing outreach throughout the affected areas. On 1 June 2020, USDA-APHIS certified that the U.S. had satisfied the World Organisation for Animal Health (OIE) criteria for eradication of virulent Newcastle disease (vND) from poultry as defined by the OIE and exhibition birds. All necessary actions and surveillance requirements were completed in accordance with the OIE Terrestrial Animal Health Code with no further detections of the disease.

Over the course of two years, more than 8,700 premises were quarantined, and disease surveillance staff made more than 226,000 premises visits and coordinated more than 11,000 laboratory submissions. Approximately 2,400 premises with a total of more than 1.2 million birds were depopulated, either because they were infected with vNDV or because they were linked to an infected premises. At the peak of the outbreak, USDA had more than 150 personnel deployed, including rotating Incident Management Team staff to assist CDFA, for a total of more than 300 State and Federal personnel responding to this outbreak. To guard against the risk of subsequent outbreaks, CDFA and USDA in partnership with many bird enthusiasts in Southern California committed to on-going monitoring for disease and continual support for biosecurity training. All backyard and exhibition poultry owners and commercial operations are encouraged to practice biosecurity measures to help prevent the introduction of disease when people enter or depart the premises, to routinely check birds for signs of illness, and to report any incidence of suspected vND or other bird diseases.

Table 1. Number of vND confirmed and presumptive positive premises, by county and dates of earliest confirmation in each county through 25 February 2020.

County	Confirmed/Presumptive Premises	Earliest Confirmation Date in County
Los Angeles, CA	50	17 May 2018
San Bernardino, CA	176	24 May 2018
Riverside, CA	269	30 June 2018
Ventura, CA	1	15 August 2018
San Diego, CA	1	31 August 2019
Alameda, CA	1	13 March 2019
Utah, UT	1	17 January 2019
Coconino, AZ	1	1 April 2019
Total	496 ⁴	

⁴ Some premises were infected twice. The total number of confirmed and presumptive premises represents the total number of unique premises affected.



Figure 1. Counties with confirmed findings of vND from 17 May 2018 to 25 February 2020.

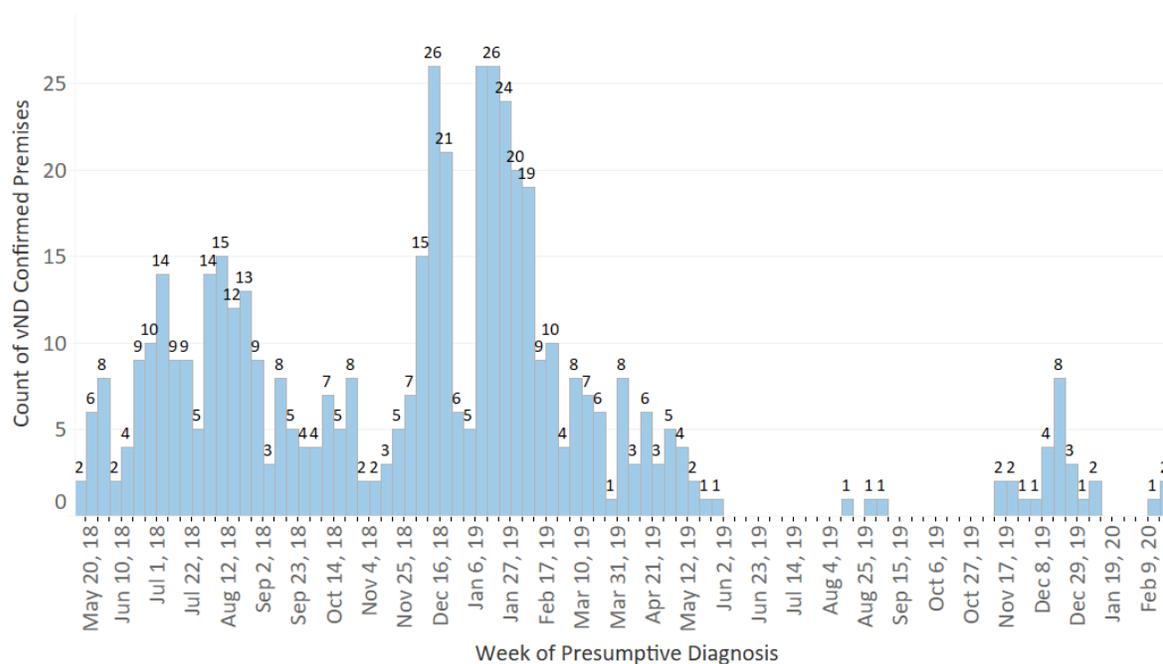


Figure 2. California vND weekly case detection curve based upon the date the case definition⁵ was met for a presumptive positive flock, by day from 17 May 2018 to 25 February 2020.

C. Surveillance Overview

Based on laboratory submission data, most premises sampled during the outbreak were small operations. A small number of these premises had both pet birds as well as chickens and less than 50 premises had only pet birds. Approximately 25 percent of the premises had two or more poultry species⁶ present at testing, with some having up to 9 different species present. Most infected, depopulated premises only had poultry species present (98 percent), with the number of species present ranging from 1 to 8 species. Thirty-six live bird markets were sampled during the outbreak.

⁵ Case definitions

- Suspect case: domesticated bird or flock having clinical signs compatible with vND; or detection of APMV-1 by rRT-PCR; or epidemiological information indicating exposure to vNDV
- Presumptive positive case: a suspect case with detection vNDV by the fusion-target rRT-PCR test at a laboratory designated by the Secretary of Agriculture
- Confirmed positive case: domesticated bird or flock from which vNDV has been identified at the NVSL as presumptive positive with confirmation of multiple basic amino acids (either directly via protein or by deduction through sequencing) in the fusion gene at the C-terminus of the F2 protein and phenylalanine at residue 117, which is the N-terminus of the F-1 protein. The term 'multiple basic amino acids' refers to at least three arginine or lysine residues between residues 113 and 116; and/or the vNDV has an intracerebral pathogenicity index (ICPI) in day-old chicks (*Gallus gallus*) of 0.7 or greater.

⁶ Includes Chickens, Chukar, Doves, Duck, Emu, Geese, Guinea Fowl, Ostrich, Peafowl, Pheasant, Pigeons, Quail, Squab, Swans, and Turkeys

Forty-five commercial and independent (larger backyard non-commercial) poultry premises⁷ within the RQA had birds and were sampled during the outbreak as part of surveillance and permitted movement testing⁸. This included 10 infected commercial and larger non-commercial poultry premises and 35 uninfected premises. An additional 15 commercial or independent (larger backyard non-commercial) premises were sampled during the outbreak but not located within the RQA.

Table 2 provides an overview of the number of laboratory accessions and samples collected from infected commercial and independent premises in the RQA by production type through 8 June 2020. Table 3 provides an overview of the number of laboratory accessions and samples collected from uninfected commercial and independent premises in the RQA by production type through 8 June 2020.

Table 2. The number of distinct sampled infected commercial and independent premises, laboratory accessions, and samples collected from infected premises in the RQA as part of vNDV surveillance by production type.

Production Type	Premises	Accessions ¹	Samples ³
Commercial Table Egg Layer	3	136	803
Commercial Table Egg Pullets	1	1	4
Independent Table Egg Producer	6	288	1,694
Total²	10	425	2,501

¹ LMS accessions were used as a proxy for the number of testing events that occurred at a given premises.

² Does not include environmental testing performed after depopulation of infected commercial and independent premises.

³ LMS ID was used as a proxy for the number of tests conducted/ samples collected. Samples are assumed to be five bird pools.

⁷ Premises types included Commercial Broiler Production, Commercial Hatchery, Commercial Table Egg Layer, Commercial Table Egg Pullets, Commercial Turkey Meat Bird, Independent Table Egg Producer, Independent Table Egg Pullets, and Squab Producer

⁸ Commercial and backyard non-commercial poultry premises within the RQA were subject to routine, ongoing active surveillance testing throughout the duration of the outbreak. Commercial and larger non-commercial, backyard premises outside the RQA with links to premises within the RQA were tested as well.

Table 3. The number of distinct sampled uninfected commercial and independent premises, laboratory accessions, and samples collected from negative premises in the RQA as part of vNDV surveillance by production type.

Production Type	Premises	Accessions ¹	Samples ²
Commercial Broiler Production	7	116	690
Commercial Hatchery	1	65	393
Commercial Table Egg Layer	13	870	5,220
Commercial Table Egg Pullets	5	209	1,188
Commercial Turkey Meat Bird	2	19	114
Independent Table Egg Producer	29	1,642	9,332
Independent Table Egg Pullets	2	171	1,021
Squab Producer	1	1	6
Total	60	3,093	17,964

¹ LMS accessions were used as a proxy for the number of testing events that occurred at a given premises.

² LMS ID was used as a proxy for the number of tests conducted/ samples collected. Samples are assumed to be five bird pools.

D. References

- USDA-APHIS (2018). "Epidemiologic Analysis of Virulent Newcastle Disease in Backyard Birds in California, December 2018." USDA:APHIS:VS:STAS:Center for Epidemiology and Animal Health. Fort Collins, CO. December 2018. M&M Doc #447.0718. 41pgs.
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II. PHYLOGENETIC ANALYSIS AND DIAGNOSTICS

A. Virulent Newcastle Disease Virus

This section describes viruses characterized from the 2018-2019 vND events in California (CA2018). The index case is chicken/California/18-016505-1/2018, which has an amino acid cleavage site of PGGRRQKR/FVGAI. The intracerebral pathogenicity index (ICPI) conducted on selected isolates in accordance with OIE guidelines confirms virulence⁹. Chickens have predominantly been affected; other species from which the virus has been recovered include turkey, peafowl (peacock), duck, goose, dove, and pigeon. Studies with the CA2018 index virus at the Southeast Poultry Research Laboratory suggest that it is highly adapted to and very infectious for chickens, and that knowledge from studies conducted on related viruses from California 2002 may be useful (Ferreira et al, 2019).

Methods

Genetic sequence data from the virus are used to determine the cleavage site, which serves as disease confirmation. Additionally, full genomic sequence data are generated and analyzed to monitor virus evolution and to inform epidemiologic investigations. Genetic data are also used to confirm that diagnostic assays are fit for purpose.

Results

NOTE: The outcomes of phylogenetic analysis should be interpreted in context of all available virus and epidemiologic information and should not be used directly to infer transmission.

The CA2018 virus (genotype Vb) is related to older Mexican-lineage viruses from Central American village poultry (Belize 2008, Honduras 2007), and the U.S. (smuggled parrot 1996, CA2002), which represent viruses from birds with low or no vaccine coverage. The current genetic analysis based upon 446 full genome sequences supports a recent, single introduction into California followed by secondary spread. Lack of epidemiologic data regarding the index premises, and of contemporary sequence data contribute to the uncertainty surrounding the origin of the outbreak. Evolutionary analysis of available sequences with the CA2018 and CA2002 viruses suggests that, although the viruses are related to one another, CA2018 is not directly descended from CA2002, and that the virus has likely been actively circulating and evolving at an expected rate; however, where and in what type of chicken population remains unclear.

There have been no changes in the amino acid profile at the cleavage site (RQKR/FVGAI) among sequenced viruses; however, synonymous nucleotide substitution within the cleavage site has been observed. The ICPI ranges between 1.6-1.8 for viruses tested (n=14).

⁹ The World Organisation for Animal Health (OIE) defines Newcastle disease as an infection with a virulent APMV-1 virus (vNDV) characterized by either an intracerebral pathogenicity index (ICPI) of 0.7 or greater in day-old chickens, or the presence of multiple basic amino acids at positions 113- 116 of the C-terminus of the fusion (F2) protein (either arginine (R) or lysine (K)), plus phenylalanine (F) at residue 117 of the F1 protein.

Divergence of the virus into two sub-groups appeared early on and, where epidemiologic data is available, has been useful to gain insights on virus spread. Two viruses that share the same nucleotides as a related reference virus at specific sites in the fusion gene represent the oldest viruses in terms of viral evolution (vNDV-00). The main sub-groups (vNDV-01 and vNDV-02) are defined by the presence of a sustained change (single nucleotide polymorphism [SNP]) in the fusion gene compared to a related reference sequence; both sub-groups have been detected in backyard, exhibition, and layer flocks (group vNDV-01 in San Bernardino, and vNDV-02 in Riverside, as well as exhibition flocks in Utah and Arizona). Further sub-clusters have also been defined by sustained SNPs along the genome.

Although geospatial clustering of viruses has been observed, the presence of different virus sub-groups in each of the major affected areas indicates virus movement within, and between, affected areas (Figure 3). Virus from affected layer facilities in Riverside County (vNDV-01) are different from those in affected layer facilities in San Bernardino County (vNDV-02) representing separate events by county; the potential for limited lateral spread cannot be distinguished from common exposure within each county based upon available data.

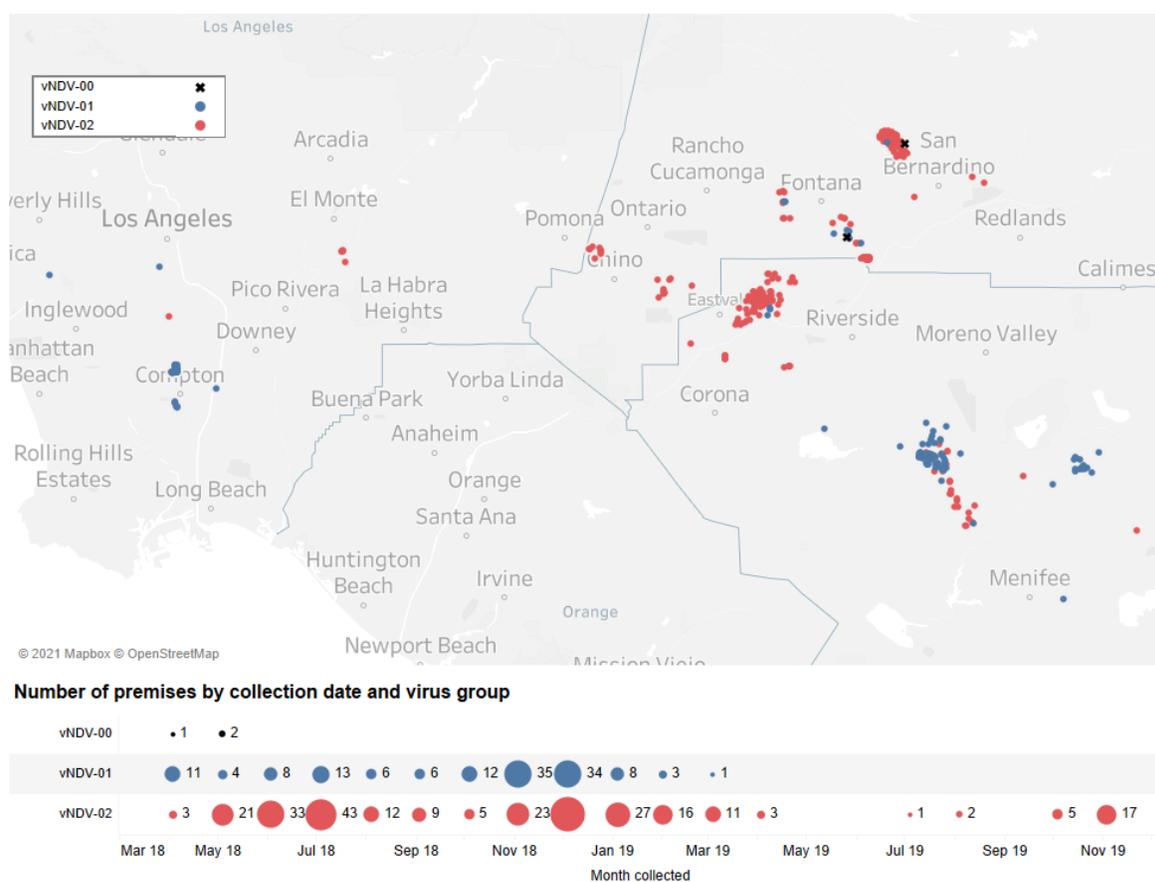


Figure 3. Distribution of analyzed viruses by virus group and date of sample collection through November 2019.

Further subgrouping within each group (vNDV-01 and vNDV-02) was observed as the outbreak continued. The presence of specific sustained nucleotide changes allows the ability to track specific

viruses (note this data should not be interpreted as a change in virulence or transmissibility). Based upon these sustained changes, the potential for epidemiologic links was further investigated for one vNDV-02 subgroup (henceforth “RV-a” for ease of reference). From 19 December 2018 to 11 May 2019, the RV-a subgroup was confirmed on 28 premises (Figure 4): 24 backyard producer premises in Riverside (n=10) and San Bernardino (n=14) counties; 2 independent table egg producer premises in San Bernardino County; 1 backyard producer premises in Compton, Los Angeles County; and 1 backyard producer premises in Flagstaff, Coconino County, AZ.

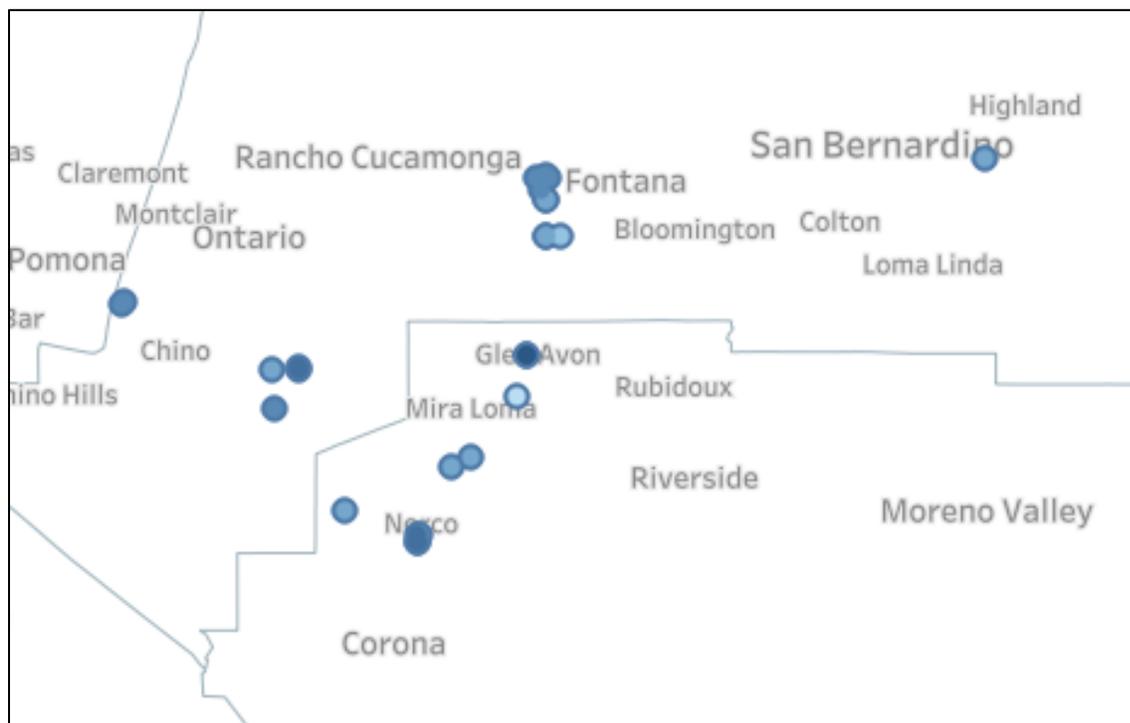


Figure 4. Distribution of the RV-a vNDV subgroup in California from 19 December 2018 to 11 May 2019. Color changes from lighter blue to darker blue over time (i.e., lighter blue dots detected earlier). Not pictured: a backyard producer premises in Flagstaff, Coconino County, AZ, (collected on 28 March 2019), and a backyard producer premises in Compton, Los Angeles County, (collected on 15 February 2019).

The city location of premises and date sampled for the RV-a subgroup are listed in Table 4. The first virus from the RV-a subgroup was detected 19 December 2018 on the premises of a backyard producer with 30 birds (21 laying hens and 7 roosters) in Riverside County. The owner called to report sick birds on 18 December 2018 reporting mortality and sick birds in their flock. The flock was depopulated on 20 December 2018. The next premises to be identified with this subgroup was a backyard producer with 320 birds in Fontana, San Bernardino County who reported sick and dead birds on 16 January 2019. Epidemiologic links between these two premises were not identified, and no recent new birds or visitors with birds were reported on the second premises. Remaining live birds on the premises were depopulated on 19 January 2019.

Co.	City	Date sample was collected (mm/dd/yy)																							
		12/19/18	01/16/19	02/12/19	02/13/19	02/14/19	02/15/19	02/17/19	02/19/19	02/20/19	02/28/19	03/03/19	03/04/19	03/12/19	03/13/19	03/17/19	03/27/19	03/28/19	03/31/19	04/01/19	04/03/19	04/08/19	04/13/19	05/06/19	05/11/19
RV	Riverside	1																						1	1
	Mira Loma			1					1																
	Corona								1																
	Norco															1			1	1	1				
SB	Fontana		1		1	1		1	1				1		1			2							
	Highland							1																	
	Ontario									1		1										1	1		
	Chino										1			1											
LA	Compton						1																		
CO	Flagstaff																1								

Table 4. Detection timeline for the RV-a vNDV subgroup in California between 19 December 2018 to 11 May 2019 by sample collection date. RV = Riverside County, CA; SB = San Bernardino County, CA; LA = Los Angeles County, CA; CO = Coconino County, AZ.

During the month of February 2019, 4 additional background premises were identified in Fontana with the RV-a subgroup: 2 in Mira Loma, 1 in Highland, and 1 in Corona (all in Riverside and San Bernardino counties). The RV-a subgroup virus was also identified on a backyard bird premises in Los Angeles County. The owner made the sick call on 2 February 2019 after a 2-day history of bird illness, reporting signs consistent with vND. An epidemiological interview was not available for this premises at the time of this analysis. There have been no other detections of the RV-a subgroup in Los Angeles County as of 23 July 2019.

The RV-a subgroup was detected in a sample from an independent table egg producer premises collected on 28 February 2019. The closest backyard premises infected with the RV-a subgroup at that time was about 2 km away. An interview was conducted with the owner of birds on the backyard premises on 24 February 2019, and no illness was reported in birds at that time. The birds were still reported to be without clinical signs on 4 March 2019 when follow-up targeted surveillance was performed, and positive results obtained. The RV-a subgroup was also confirmed in two additional backyard premises in Chino and one in Norco in early March. In early April, two additional premises were identified in Ontario (approximately 1.4 km from the premises sampled on 28 February 2019) and three additional premises were detected in Norco in Riverside County.

A second independent table egg producer premises in San Bernardino County was identified with the RV-a subgroup from a sample collected on 12 March 2019. Surveillance in the area around this operation found several backyard premises in proximity that were also infected with the RV-a subgroup. Of these, one backyard premises had reported clinical signs consistent with vND within the previous 30 days. In the epidemiologic report for this backyard premises, the interviewer noted that the birds were loose and known to wander onto the adjacent neighboring independent table egg producer premises. Two additional infected backyard premises were identified nearby during late March.

A single exhibition premises was identified with the RV-a subgroup in Flagstaff, AZ based on samples collected on 28 March 2019. Although no epidemiologic links were identified, the virus data connects this detection to other infected premises in California. No further cases in AZ have been detected.

No presumptive or confirmed positive premises were reported between the weeks of 2 June 2019 and 10 August 2019. On 14 August 2019, a new premises was confirmed in a feed store within a previously infected control zone through outreach testing. Further surveillance identified additional infected backyard premises within the control zone. Although there was a period of approximately 10 weeks where no virus was detected, genetic analysis of the new detections was closely related to a specific subgroup of vNDV-02 viruses that survived eradication attempts. This cluster of detections starting August 14 all fell into the same vNDV-02 subgroup and did not represent a new introduction of the virus, based upon molecular studies and epidemiologic investigation (Figure 5).

Examination of this subgroup of the vNDV-02 viruses provided valuable information on disease risks and linkages, while also revealing the complex and poorly understood pathways of transmission in these populations. Spatial and temporal patterns of this virus subgroup highlight the interconnected nature of these neighborhoods and populations, which can complicate disease control efforts. Phylogenetic analysis represents an important tool for understanding disease spread, and this understanding is further enhanced where epidemiologic data are available.

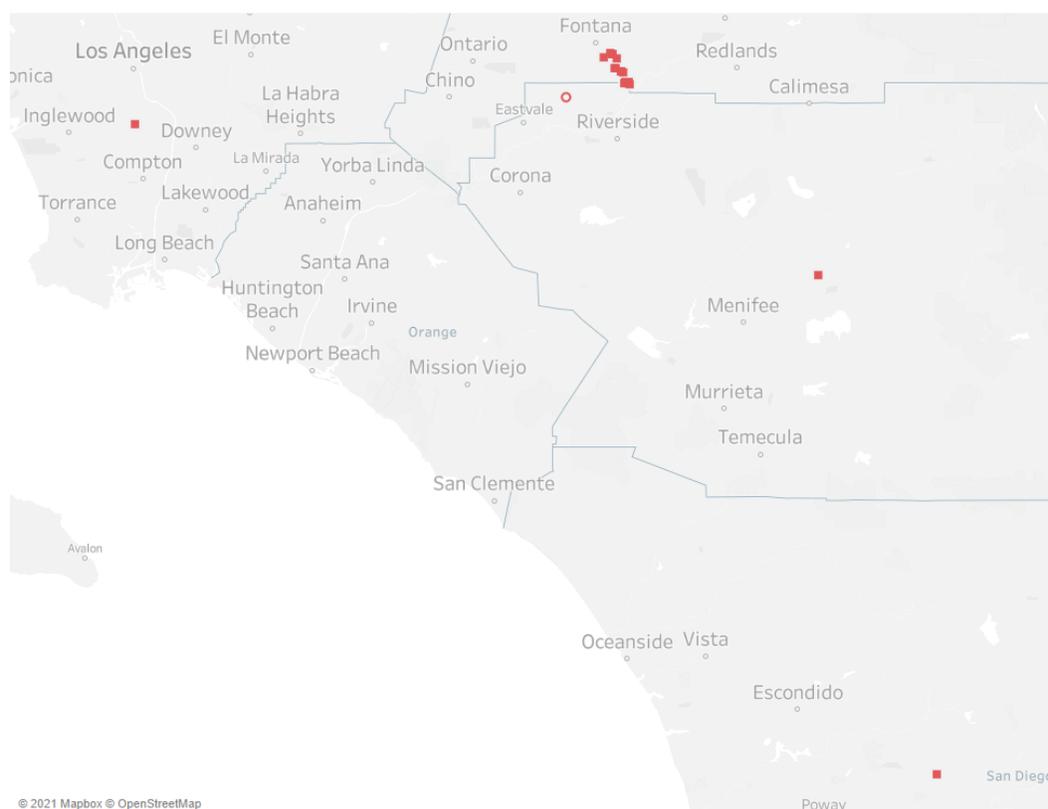


Figure 5. Distribution of subgroup of vNDV-02 viruses which occurred after 1 August 2019. The red circle indicates the location of an earlier detection of this virus subgroup prior to its resurgence in August.

B. Comparison to Other Viruses/Lineages

The CA2018 virus is not related to classic Newcastle disease vaccine strains, nor to available strains from vaccinated poultry in Mexico (2000-2010). CA2018 is also unrelated to viruses endemic to columbids (pigeons, doves; genotype VI), as well as genotype V from double-crested cormorants (Figure 6).

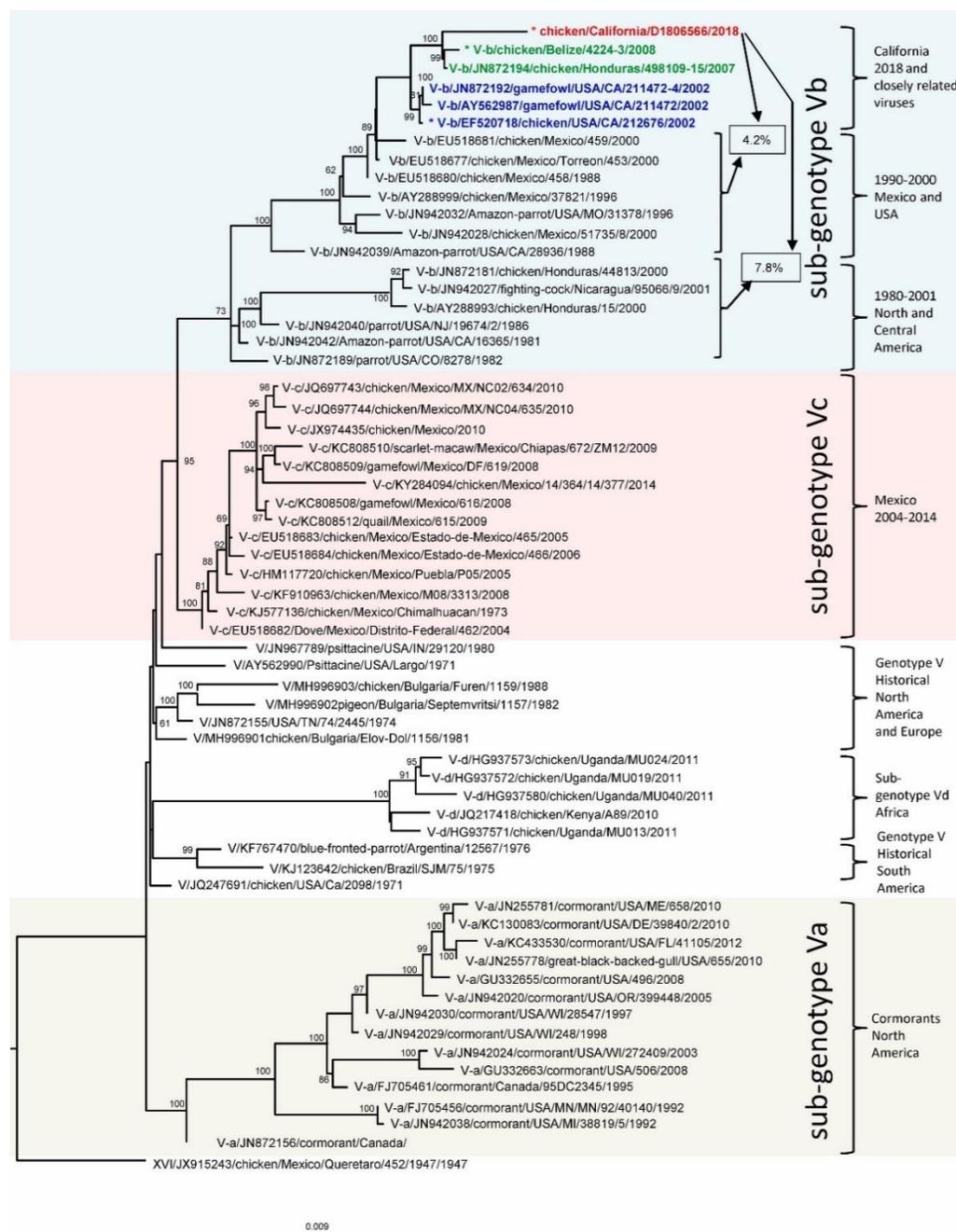


Figure 6. Phylogenetic analysis based on the full-length nucleotide sequence of the fusion gene of isolates representing Newcastle disease virus class II, genotype V (Dimitrov et al., 2019)

C. Diagnostics

Testing avian swabs/tissues for APMV-1 involves screening assays (real time reverse transcription polymerase chain reaction [rRT-PCR]), virus isolation, and characterization of the virus (sequencing and in vivo tests). The National Animal Health Laboratory Network (NAHLN) tests swab/tissue first by the APMV-1 matrix-target rRT-PCR test,¹⁰ best suited to detect Class II viruses that contain low and highly virulent pathotypes, including vaccine viruses. Detections by the matrix-target test are subsequently tested by a fusion gene-target rRT-PCR test, which is designed to allow rapid identification of virulent viruses reportable in poultry. This approach does not provide the genetic or geographic lineage of the virus. Sequence analysis of the virus compared to the assay primers and probes confirmed high identity between the CA2018 virus sequences and the fusion gene-target rRT-PCR test. A negative fusion gene-target test in the face of clinical signs requires further testing including virus recovery, sequence, and/or ICPI testing.

Under normal surveillance, all poultry samples with a nonnegative test result by APMV-1 PCR or virus isolation are forwarded to NVSL for confirmatory testing; for the current CA vND event, the NAHLN laboratory is using the highly matched fusion-target assay. The NVSL uses Sanger sequencing protocols to generate partial fusion gene sequence directly from the sample for virulence determination, where sufficient viral RNA is present. Whole genome sequencing is conducted on all isolated viruses, and select viruses are further characterized by ICPI in specific pathogen-free chickens.

The NVSL confirms the virus lineage and virulence through molecular sequencing. Where no virus can be recovered nor sequence obtained directly from sample(s), the virulence is determined by the clinical presentation of the flock compared to the USDA vNDV case definition.

D. References

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¹⁰ PCR results from the NVSL are reported as “detected” or “not detected” and include the cycle threshold (Ct) value. The lower the Ct value, the more viral nucleic acid was detected.

III. POPULATION AT RISK

A. Predicting Areas of Backyard Bird Ownership

The distribution of backyard bird flocks in the United States is currently unknown. However, statistical modeling can be used to estimate the likely locations and densities of backyard flocks in a given geographic area using socio-economic and demographic variables that have been shown historically to be related to bird ownership. This approach was used to develop neighborhood-level risk maps to identify and prioritize areas for surveillance during the 2002-2003 outbreak of vND in the United States (Freier et al., 2004, Freier et al., 2007). Building on that historical work, statistical models were developed that identified areas with increased probability of backyard poultry ownership to inform surveillance and response efforts. The objectives of this analysis were two-fold, first to identify socio-economic and demographic risk factors associated with poultry ownership. Second, to use these relationships to predict the number of backyard poultry flocks at the neighborhood-level.

Methods

Data

Census block groups within 1.5km of positive premises were identified. These block groups, which can be conceptualized as neighborhoods, are assumed to have been completely surveyed for presence of poultry. The number of premises with poultry were tallied for each block group. These counts of homes with poultry were used as the response variable in models. The total number of households in each block group were extracted from the census data and assumed to represent all possible households that could have had poultry. Census variables previously identified as associated with backyard poultry ownership were considered in models determining associations among poultry ownership and socio-demographic and economic variables.

Model

Generalized additive models (GAMs) accounting for correlation in spatial areal units and non-linear effects of predictors were used to analyze vND outbreak response data and predict the number of backyard flocks present in census block groups. Three alternative model structures (Poisson, Binomial, and Negative Binomial) were evaluated based on predictive capacity as measured using adjusted R^2 and amount of deviance in the data the model explained, and Akaike information criterion (AIC). Because of non-linear distributions of predictors, a thin plate spline smoother was used for each predictor. The appropriate smoothness for each predictor was determined using likelihood-based methods with a smoothing penalty. The smoothing penalty, which includes a small shrinkage component, results in smoothing parameters that become identically zero when the predictor is not significant. This allows smoothing parameter selection to effectively remove the predictor from the model altogether without requiring formal model select methods which are CPU-intensive. Models were validated using k-fold cross validation consisting of 10 bins. Models were fit using the mgcv package in R (R Core Team, Vienna Austria, 2020).

Results

The best predictive model was a generalized additive model (GAM) accounting for correlation in spatial areal units and non-linear effects of predictors. The model was used to predict the number of backyard

flocks present in each census block group. The GAM used a Negative Binomial error structure in the response variable (number of backyard flocks in each census block group). Because of non-linear distributions of predictors used in the GAM smoothing splines were used for predictors that demonstrated non-linear associations with the response variable. The appropriate smoothness for each predictor was determined using likelihood-based methods with a smoothing penalty.

The final model explained 76.3 percent of the deviance. Out-of-sample cross validation using k-fold methods, had high correlation (0.75) among out-of-sample and predicted number of poultry flocks in census block groups (Figure 7). These results provide confidence in the predicted number of backyard flocks in census block groups. Despite this confidence there was large variation in the predictions thus predictions should be interpreted as relative measures of backyard poultry ownership with areas of high predicted backyard poultry flocks indicating relatively greater numbers of flocks.

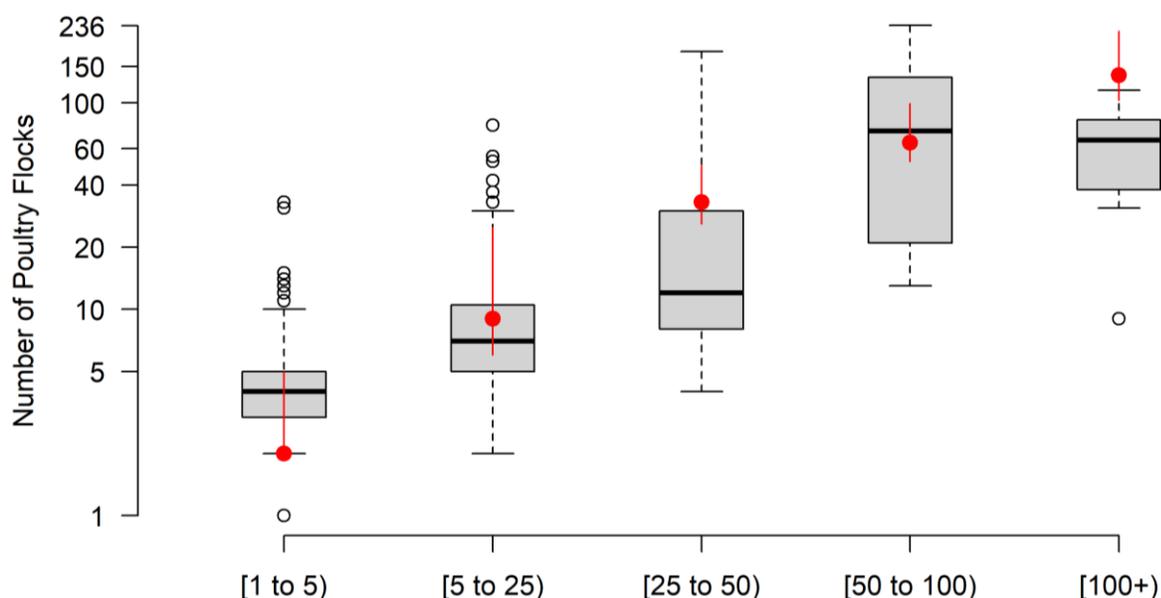


Figure 7. Comparison of predicted number of backyard poultry flocks (boxplots) with observed distribution of backyard poultry flocks (red). The model tends to over-predict for very small numbers of poultry flocks (<5) in census block groups and under-predict for block groups with very large numbers of poultry flocks (>100).

Socio-demographic variables associated most closely with poultry ownership were the level of education of the population and the housing density (Figure 8 and Figure 9). Additionally, the number of nonfamily members living in homes and the degree of ethnic diversity in the neighborhood were also associated with poultry ownership. Socio-economic factors of household income, home value, and receipt of public assistance were important but less so than other factors. Home density, total population, and the mean age of homes in years were also important predictors.

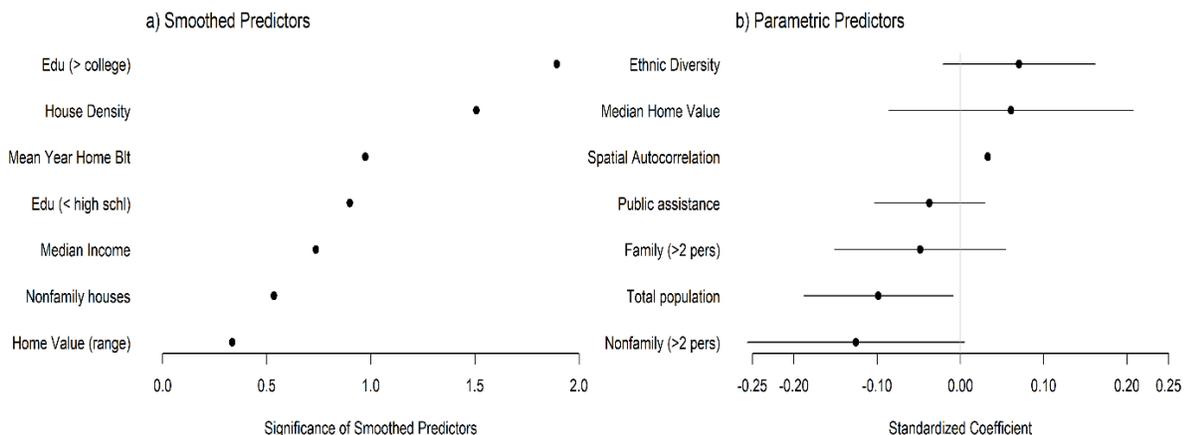


Figure 8. Predictive variables used in the generalized additive model. Panel A presents the significance of smoother (non-linear) predictors and Panel B presents the estimated effect size for the parametric predictive variables.

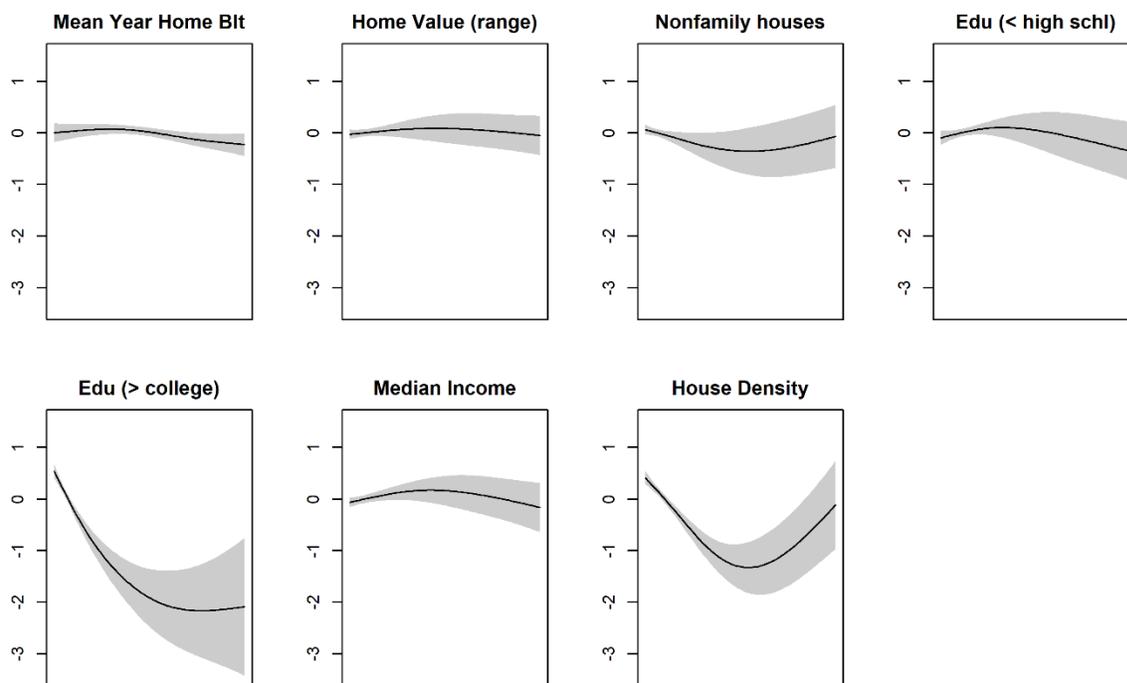


Figure 9. Smoothing splines describing the relationship between number of poultry flocks and non-linear predictor variables.

The geographic distribution of poultry ownership (Figure 10) aligns well with the observed geographic distribution of poultry flocks. Additionally, this map identifies populations of backyard poultry that may be important for surveillance or at risk for vNDV transmission. These predictions can be used to inform surveillance activities, planning related to the number of vaccine doses that may be needed, or the number of poultry that may need to be culled to control transmission and spread. Additionally, these predictions also identify populations of poultry owners that may be important to target for education campaigns or other proactive prevention activities

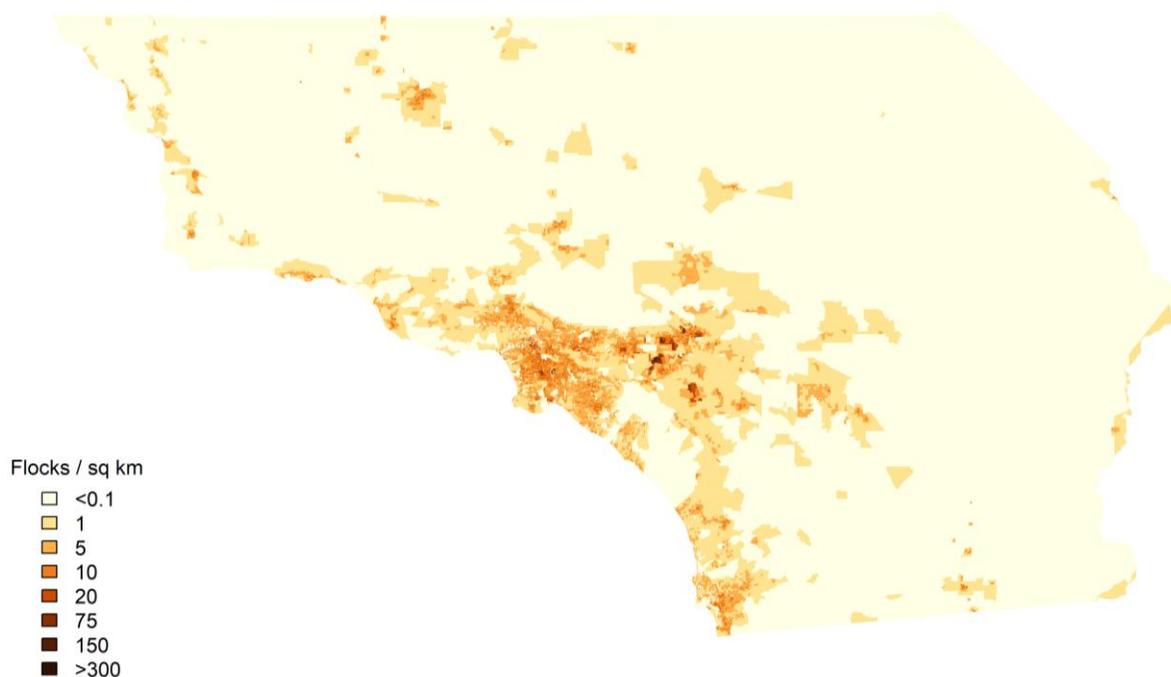


Figure 10. Predicted geographic distribution of backyard poultry flocks in southern California. The map presents the density of flocks per km².

Summary

The area impacted by the outbreak had a very dense population of backyard poultry. However, limited information was available prior to the outbreak to aid responders in locating and testing backyard poultry operations. Using sociodemographic variables to predict where backyard poultry are likely to be kept provided rapid information that served as a baseline for response planning. This work built upon analyses that were performed during a previous outbreak of vND in 2002-2003. While many of the same predictors were found to be significant, the resurgence in popularity of keeping backyard chickens has led to the presence of backyard poultry in areas where it was historically uncommon. As a result, model outcomes should be interpreted as relative measures of backyard poultry ownership and supplemented with on the ground surveillance and neighborhood visits.

IV. EPIDEMIOLOGIC RISK

A. Case Control Study

A case-control epidemiological analysis was performed on confirmed and presumptive positive virulent Newcastle disease (vND) backyard/exhibition premises, dangerous contact premises, and noninfected premises. Data were obtained from in-person interviews using the CDFA Non-Commercial Premises Virulent Newcastle Disease Epidemiology Questionnaire (Appendix A: Questionnaire). Questionnaire data were entered into the USDA's Emergency Management Response System (EMRS).

Methods

Data were analyzed for 912 premises: 137 confirmed or presumptive positive premises, 68 dangerous contact premises, and 712 noninfected premises. The analysis included data from questionnaires that were completed from 16 May to 9 November 2018 and includes all confirmed and presumptive premises for which questionnaire data were entered into the EMRS as of 9 November 2018. The questionnaire form was updated in July 2018 with additional questions; 69 respondents completed the original questionnaire and 848 completed the updated questionnaire. Questionnaires were not complete for all premises, such as in cases in which the owners refused to provide answers to certain questions. The number (n) of responding premises is noted in Table 5. Odds ratios, p-values and 95-percent confidence intervals (CI) for flock characteristics and other risk factors were estimated by univariate logistic regression, using confirmed/presumptive premises as cases and noninfected premises as controls. Dangerous contacts were excluded from the regression analysis. To identify significant risk factors, while controlling for possible confounding variables, two multivariable logistic regression analyses were performed. The first included questions found in both versions of the questionnaire, while the second included questions found only in the newer version of the questionnaire. All variables that had a statistically significant (p-value < 0.1) predictive effect on being a case were included in the analysis, and backward stepwise elimination was used to obtain final models.

Results

Premises characteristics

The reported flock sizes ranged from 1 to 853 birds (mean=51, median=18 birds). Thirty-three percent of all backyard/exhibition flock owners had multiple bird species on their premises. These premises primarily had backyard chickens (82.9 percent). Fewer premises had exhibition birds/game fowl (8.5 percent), and ducks/geese (11.5 percent). Other types of birds were reported on 30 percent of premises; the most commonly reported species were pigeons, turkeys, peafowl, parrots, and cockatiels. Besides birds, 35 percent of owners had other livestock species on their backyard premises, 76 percent had dogs/cats, and 8.5 percent reported other non-bird species.

Housing types

Most respondents (59.6 percent) reported housing birds outdoors in cages or coops, with 28 percent housing birds outdoors in open top pens or enclosures, and only 7 percent housing birds indoors. Only 5 percent of respondents reported keeping birds individually tethered, and 35 percent reported having free-range birds.

Illness and mortality

Sixty-four percent of case premises reported bird illness, and 65.7 percent reported mortality. The mean time reported between onset of illness and presumptive detection was 9.6 days (median=6.0, range 1-90 days). The mean time between onset of mortality and presumptive detection was 10.1 days (median=4.7 days, range 1 to 90 days). As an indicator of background morbidity and mortality, the percentage of control premises reporting illness was 10.5 percent and mortality was 17.6 percent.

Risk factors – Univariate analysis

- Flock size

Case premises reported larger flock sizes than control premises. The odds of being a case were significantly greater for flock sizes greater than 100 birds (OR = 11.6, 95 percent CI: 6.4-21.0) or from 20 to 99 birds (OR = 5.0, 95 percent CI: 3.0 – 8.5) when compared with flock sizes of fewer than 20 birds.

- Bird types

Case premises were more likely to report having flocks that included exhibition birds or other non-chicken bird species than control premises (OR = 7.7, 95 percent CI: 4.6-12.8).

The odds of becoming a case premises were also higher when roosters comprised more than 50 percent of the adult birds in the flock (OR=4.3, 95 percent CI: 2.7-6.7).

- Contact with other domestic and wild birds

Although only 8.8 percent of case premises reported keeping birds at other premises, the odds of being a case were higher (OR = 3.9, 95 percent CI: 1.8-8.2) when birds were kept at multiple locations.

A high percentage of both case and control premises reported having neighbors with birds (75.9 percent and 55.0 percent, respectively); however, premises that reported that their birds visit neighboring properties or that their neighbors' birds visit their property did not have increased odds of becoming a case. Contact with wild birds (OR = 3.5, 95 percent CI 2.0-6.2) was associated with greater odds of becoming a case premises.

- The use of Newcastle vaccine

The percentage of premises reporting the use of Newcastle vaccine was low overall (6.9 percent). The percentage of case premises that reported using Newcastle vaccine was much higher than the percentage of controls that reported using Newcastle vaccine (18.9 percent vs 5.6 percent, respectively), and the risk of disease was greater among flocks that reported use of Newcastle vaccine (OR = 4.2, 95 percent CI: 2.4-7.5).

Risk factors – Multivariate analysis

Many of the risk factors described previously are related. A multivariate analysis was performed in order to provide adjusted odds ratios for risk factors, while considering the interrelationships among these flock management characteristics and behaviors. For the multivariate analysis including both versions of the questionnaire, 103 cases and 579 controls were examined (Table 6). Larger flock sizes (OR = 3.9, 95

percent CI: 2.2-7.1 for 20-99 birds; and OR = 5.7 95 percent CI: 2.8-11.7 for flocks with more than 99 birds), the presence of game fowl on the premises (OR = 4.6, 95 percent CI 2.5-8.6), and having greater than 50 percent of adult birds as roosters (OR = 2.4, 95 percent CI 1.4-4.1) significantly increased the odds of becoming infected. A nested analysis looking only at questions found in the newer version of the survey (84 cases and 622 controls) identified these same factors, as well as wild bird contact with domestic birds (OR = 3.1, 95 percent CI: 1.7-5.9), and having neighbors with birds (OR = 2.2, 95 percent CI: 1.2-3.9) as significant risk factors.

Summary

These results suggest that flock size, ownership of exhibition birds, a high proportion of roosters in the flock, and housing that facilitates contact with nearby domestic and wild birds are risk factors for vND infection in this population. Some of these practices have been shown to be risk factors in other studies or previous vND outbreaks in the United States, as summarized below. However, not all epidemiology questionnaires were complete, and it is likely there is misclassification bias for some of these results, such as the type and number of birds on premises, the number of owners, and use of Newcastle vaccine; therefore, results should be interpreted with caution.

Summary of Historical Epidemiologic Risk Factors

An epidemiological study of backyard premises during the 2002-2003 California vND outbreak identified the following risk factors for vND infection on premises: presence of game fowl, presence of feral chickens, flock sizes larger than 40 birds, and multiple owners of a flock. Epidemiological descriptions of infected backyard premises in the 1971-1974 outbreak identified contact with infected commercial layer farms as the primary source of infection, followed by active trading of birds among backyard flocks and purchases of infected exotic birds from dealers. The severity of infection among commercial premises during the 1971-1974 outbreak was attributed to the high density of egg-laying premises and extensive contact among those premises. In both the 1971-1974 and 2002-2003 California vND outbreaks, a suspected risk factor for vND infection in commercial premises was movement of contaminated equipment, such as egg carts.

Table 5. Characteristics of backyard case premises (confirmed/presumptive positive for vND), dangerous contact (DC) premises, control premises (C), and odds ratios (OR) and p-values calculated by univariate logistic regression (dangerous contacts excluded).

Characteristic	Level	n			OR	p-value
		Case	DC	Control		
Number of birds	1-19	20/125	8/43	385/698	Ref	
	20-99	64/125	9/43	245/698	5.0	<0.001
	100+	41/125	8/43	68/698	11.6	<0.001
Bird species on premises	Backyard chickens	98/125	23/25	635/695	0.34	0.207
	Exhibition birds	37/125	5/25	36/695	7.7	0.005
	Ducks/geese	18/125	5/25	82/695	1.3	0.329
	Other species	45/125	8/25	217/695	1.2	0.019
Adult birds >50 percent roosters		43/103	3/23	84/583	4.3	0.001
Owners keep birds on other premises		12/119	1/23	19/676	3.9	<0.001
Nonbird species or wildlife on premises		17/67	3/9	38/193	1.4	0.328
Housing	Inside home	3/93	0/19	52/671	0.4	0.126
	Outdoor open top	34/93	10/19	217/671	1.2	0.417
	Outdoor cage/coop	76/93	13/19	458/671	2.1	0.009
	Individual tether	6/93	0/19	43/671	1.0	NA
	Free range	46/93	10/19	266/671	1.5	0.072
Movement of new birds onto the premises within 30 days prior to the interview		12/121	2/24	44/674	1.7	0.123
Movement of birds off the premises within 30 days prior to the interview		8/119	0/23	30/651	1.5	0.33
Give/sell eggs		11/92	2/19	79/662	1.0	NA
Neighbors have birds		66/87	13/19	357/649	2.6	<0.001
Birds visit neighbors		16/88	2/18	392/624	1.8	0.063
Wild birds have contact with domestic birds		77/92	16/20	392/660	3.5	<0.001
Newcastle disease vaccine	No	84/122	22/25	589/683	Ref	
	Yes	23/122	2/25	38/683	4.2	<0.001
	Unsure	15/122	1/25	56/683	1.9	0.776

Table 6. Adjusted odds ratios (OR) for significant risk factors identified in multivariate regression analyses.

Characteristic	Level	OR	p-value
Number of birds ¹	1-19	Ref	
	20-99	5.4	0.04
	100+	9.0	0.001
Game fowl on premises ¹		4.8	0.001
Adult birds >50 percent roosters ¹		2.4	<0.001
Neighbors have birds ²		2.2	0.007
Wild birds have contact with domestic birds ²		2.6	0.003

¹Results from analysis that included questions found on both versions of the survey (102 cases and 538 controls)²Results from analysis that included questions found only on the newer version of the survey (84 cases and 604 controls)

B. Epidemiologic Investigation of vNDv-Infected Commercial and Backyard Non-Commercial Laying Chicken Premises

Between 14 December 2018 and 20 March 2019, one commercial table egg pullet, three commercial table egg layer, and six backyard/non-commercial layer chicken premises in Riverside County (n=5) and San Bernardino County (n=5) were confirmed positive for vND (Table 7)¹¹.

Methods

A descriptive epidemiologic study was performed on 10 vNDv-infected commercial and backyard non-commercial layer chicken premises. Twenty-eight non-infected commercial and backyard non-commercial layer chicken premises located within the three-county regional quarantine area were included in the study as controls. CDFA personnel administered an in-person questionnaire to individual(s) on each premises most familiar with its management and operations. Questions focused on management practices, biosecurity, and potential epidemiologic contacts to generate hypotheses about potential risk factors for infection with vNDv. Questionnaires were not completed for all premises, such as in cases where the respondent declined to provide answers to certain questions. The numbers of responding premises for each question are noted in

¹¹ Commercial table egg layer and commercial table egg pullet premises are defined as those with more than 75,000 birds. Table egg layer premises with fewer than 75,000 birds are referred to as backyard/non-commercial layer chicken premises.

Table 9. Hypothesis testing for all yes/no questions was performed using Fisher's exact test, given the small sample sizes; however, the resulting p-values for all questions were greater than 0.05. Therefore, odds ratios and statistical results are not provided.

Results

Case premises and control premises reported similar mean numbers of birds, numbers of flocks, numbers of houses in use, and numbers of employees (

Table 8). Transmission of vND virus in commercial flocks in previous US outbreaks has been attributed to movement of live birds, sharing of equipment, and contaminated service vehicles (Bulaga et al., 2004, Burrige et al., 1975, Utterbeck and Schwartz, 1973). Results of the questionnaire, focusing on risk factors identified in previous outbreaks, are summarized below and in

Table 9. Results showed that some factors and management practices were shared across infected farms; however, the significance of these similarities is difficult to interpret given the small number of infected farms and the study design. When considered in conjunction with knowledge of practices and risk factors from previous outbreaks, this information may provide insights into trends of management practices over time and elucidate opportunities to implement additional mitigations in the future.

Table 7. Production types, counties, confirmation dates, and numbers of euthanized birds on 10 vNDv infected commercial and backyard/non-commercial layer chicken premises.

Production Type	County	Confirmation Date	Number of Birds Euthanized
Commercial Table Egg Pullet	Riverside	14 December 2018	103,000
Commercial Table Egg Layer	Riverside	7 January 2019	140,732
Commercial Table Egg Layer	Riverside	9 January 2019	172,187
Backyard Non-Commercial Laying Chickens	San Bernardino	24 January 2019	46,953
Commercial Table Egg Layer	Riverside	1 February 2019	406,402
Backyard Non-Commercial Laying Chickens	San Bernardino	16 February 2019	71,955
Backyard Non-Commercial Laying Chickens	San Bernardino	2 March 2019	63,000
Backyard Non-Commercial Laying Chickens	San Bernardino	2 March 2019	28,000
Backyard Non-Commercial Laying Chickens	San Bernardino	14 March 2019	42,282
Backyard Non-Commercial Laying Chickens	Riverside	20 March 2019	40,542

Risk /Protective Factors

- **Ownership**
One case premises and four control premises reported having at least two collocated flocks with different owners. Eight owners of case premises and nineteen owners of control premises reported owning multiple premises.
- **Vaccination**
All cases and controls that provided an answer (10/10 and 26/28, respectively) reported that their birds had been vaccinated for Newcastle virus.
- **Use of external poultry services**
Four case premises and seven control premises reported using outside vaccination crews. Four cases premises and seven control premises reported using outside beak trimming crews. Zero case premises and four control premises reported using outside layer catch

crews. Four case premises and ten control premises reported that non-employees hauled spent hens away.

- Dead bird disposal

Six case premises and seven control premises reported composting. Two case premises and four control premises reported incinerating. Two case premises and nine control premises reported disposing in a landfill. Zero case premises and two control premises reported using a renderer. Six control premises reported other methods of disposal.

- Manure hauling

Nine cases reported using five different manure haulers. Twenty-three controls reported using ten different manure haulers.

- Sources of feed

One case reported supplying its own feed while the other nine used two different feed suppliers. Four controls reported supplying their own feed while twenty-four reported using five different feed suppliers. No cases and only two controls reported using more than one feed supplier.

- Physical biosecurity

All cases and most controls (25/26 responding) had a perimeter fence with a gate and disinfection station at the entrance. All cases and most controls (26/27 responding) restricted access to essential personnel.

Table 8. Numbers of birds, numbers of flocks, numbers of houses in use and numbers of employees reported by vND infected case premises (n=10) and control premises (n=28) located in the three-county regional quarantine area of southern California.

Characteristic	Case mean (range)	Control mean (range)
Reported number of birds	114,325 (24,000-420,000)	102,177 (740-1,500,000)
Number of flocks	4 (1-7)	3.4 (1-8)
Number of houses in use	10.4 (1-28)	7.9 (1-20)
Number of employees	10.5 (2-32)	8.3 (2-27)

Table 9. Management characteristics of vND infected case premises (n=10) and control premises (n=28) in the three-county regional control area of southern California.

Characteristic	Level or response	N cases	N controls
Production systems	Inline layers	3/10	2/28
	Offline layers	7/10	22/28
	Brooders	0/10	2/28
	Pullets	1/10	7/28
Housing type	Open sided	3/10	12/28
	Open sided with curtains	5/9	7/28
	Closed house	1/10	7/28
	Pasture raised	1/10	2/28
Raise own pullets	Yes	5/10	16/28
Buy adult hens	Yes	2/9	8/26
Live bird market supplier	Yes	0/10	2/28
Eggs processed onsite	Yes	5/10	13/25
Use processor that processes eggs for other premises	Yes	3/9	10/25
Repackage processed eggs from other premises	Yes	0/7	7/26
Equipment/vehicles shared with other premises	Feed truck	1/8	0/26
	Egg truck	2/8	3/26
	Live bird hauler	0/8	4/26
	Unspecified vehicle	1/8	7/26
	Egg flats/racks	3/8	8/26
	Fertilizer machine	3/8	3/26
Employees shared between premises	Yes	5/5	7/28
Use reusable egg flats	Yes	9/9	21/26
Transport eggs for other producers	Yes	0/9	4/24
Pullets delivered as split load for more than one premises	Yes	0/9	1/23
Consumers enter premises to purchase birds or eggs directly	Yes	4/10	9/28

Disposal of rejected eggs	Breaker plant	0/9	3/26
	Landfill	1/9	4/25
	Rendering	1/9	6/25
	Composted onsite	7/9	9/25
	Buried onsite	0/9	1/25
	Other	1/9	11/25
Perimeter fence with gate and disinfection stations	Yes	10/10	25/26
Garbage/dead bird pickup restricted to outside perimeter fence	Yes	3/9	12/26
Other species on premises	Waterfowl	1/10	2/28
	Gamefowl	0/10	0/28
	Other birds	2/10	9/28
	Hooved animals	3/10	6/28
	Dogs	5/10	13/28
	Cats	2/10	12/28
	Rodents	4/10	13/26
	Other non-birds	3/4	3/17
Free range poultry observed on premises	Yes	2/10	3/28
Free range poultry observed nearby outside premises	Yes	5/10	7/28
Backyard poultry within 0.5 miles of premises	Yes	6/10	16/28
Employees wear dedicated shoes that stay on premises	Yes	9/10	22/28
Employees wear dedicated clothing that stay on premises	Yes	6/10	17/28
Employees Reside on premises	Yes	5/10	19/27
Employees sign agreement not to own birds	Yes	10/10	26/27
Downtime required after visiting other premises with birds	Yes	10/10	26/27

C. References

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V. ESTIMATING DISEASE SPREAD

A. Flock Disease Spread Model—Early Outbreak

Methods

An epidemiologic scenario was developed in InterSpread Plus® v. 6.01.44 (Stevenson et al., 2013) to model the introduction and spread of vND from confirmed premises in San Bernardino county, California following the first case detection. Commercial and backyard farm units from the Western United States (17 states) were incorporated into the model. The Farm Location and Animal Population Simulator was used to generate likely farm locations based on geospatial characteristics, with backyard farm locations adapted from current and historic outbreak-related data. Model parameters were developed to reflect the impact of sustained outreach activities, incorporate preliminary experimental data on viral pathogenesis in chickens, include current strategies for active surveillance of commercial operations, and describe the potential geographic extent of disease spread during the silent-spread period. The model was updated regularly in order to provide timely results to the response during the early phase of the outbreak. The results presented below were developed early in the outbreak in the absence of information on risk factors. The actual outbreak data has been used to better refine and improve the model for future applications.

Results

Summary outcomes for a 300-iteration scenario were generated from ten seeded-sites. The seeded sites were based on the latitude/longitude of initial confirmed cases of vND in San Bernardino County. Simulations include control activities implemented in the vND response, including outreach, quarantine, euthanasia/depopulation of detected premises, movement controls, tracing, and active and passive surveillance. Note: These outcomes are based on a completely naïve poultry population. The variable levels of vaccination applied within backyard farms is not explicitly modeled in this scenario.

The summary of results and their potential implications for the current vND outbreak are as follows:

Initial disease spread commonly involves direct movements of infectious birds, but local spread becomes more prevalent as outbreaks become greater than 50 infected premises.

Direct contacts associated with live animal movements accounted for 36 percent of spread for simulated outbreaks that resulted in less than 50 infected premises, and 27 percent of spread for simulated outbreaks that resulted in 50 or more infected premises.

Local area spread became more prevalent as simulated outbreaks became larger, being responsible for 56 percent of disease spread for simulated outbreaks that resulted in 50 or more infected premises.

Implications: As the number of detected premises continues to increase, outcomes from simulated outbreaks suggest that local spread of vND among premises might be responsible for additional infections. Local spread is associated with distance between infectious and susceptible premises and represents mechanisms of spread that are difficult to trace, such as movement of free ranging birds, wildlife, or fence-line contact. Good biosecurity practices and measures are the best way to prevent

local spread (e.g., keeping outside birds in cages, moving cages away from neighboring fence lines, repairing damaged/missing fences, rodent control, covering/tarping cages to decrease wildlife/rodent/loose-chicken exposures, and the spread of vND viral particles into the environment).

Small backyard operations¹² are the primary premises involved in outbreaks; large backyard operations or commercial poultry farms have a lower likelihood of becoming infected.

Across all simulated outbreaks, large backyard operations represented slightly less than 1 percent of all infected premises, and commercial poultry farms represented 0.14 percent of all infected premises.

vND-infected small backyard operations in 100 percent of all simulated outbreaks, large backyard operations in 22 percent of all simulated outbreaks, and commercial poultry farms in 7 percent of simulated outbreaks.

All spread to commercial operations resulted from indirect contact (e.g., people or vehicles moving from operation to operation) with infected, primarily small backyard operations.

Implications: Unless generated by indirect contacts with infected backyard operations, outcomes from simulated outbreaks suggest a low probability of spreading vND to commercial farms.

The extent of spread for simulated outbreaks is primarily in San Bernardino, Los Angeles, and Riverside counties.

When considering disease spread within the silent period of the outbreak (three days prior to the first observation of clinical signs to the day of first detection), spread from infected premises in San Bernardino County to backyard chicken premises in Riverside County occurred in 66 percent of simulated outbreaks and to backyard chicken premises in Los Angeles County in 65 percent of simulated outbreaks.

In the current modeling scenario, 42 percent of simulated outbreaks involved 50 or more infected premises, and 19 percent of outbreaks involved 100 or more infected premises.

Implications: Simulated outbreaks suggest future detections in other Southern California counties, most commonly Riverside and Los Angeles. In addition, some infected premises might not be detected due to natural viral elimination from these premises (i.e., birds die and go unreported) and/or no new, naïve birds being brought onto previously infected premises.

¹² In the model operations are defined as follows:

- 1) Commercial poultry farms: more than 75,000 table egg laying chickens, or more than 100,000 meat-type chickens, or more than 30,000 meat-type turkeys
- 2) Large backyard operations: more than 1,000 birds but fewer than the number of birds described for commercial operations
- 3) Small backyard operations: fewer than 1,000 birds

B. Comparing Alternative Control Strategies—Mid-Outbreak

Methods

As the outbreak progressed, selected parameters were revised from preliminary scenarios described previously to compare the impact of alternative control strategies on the severity and duration of simulated vND outbreaks. This analysis was performed and supplied to the Incident Coordination Group approximately 3 months into the outbreak.

Summary outcomes for a series of four 250-iteration scenarios were generated from 57 selected sites. The initially infected sites in the model were based on the latitude/longitude of initial confirmed cases of vND in San Bernardino and Riverside counties and from premises frequently infected during the silent spread period identified in previous modeling analyses. Each simulated outbreak was allowed to run for a maximum of 365 days from detection of the first infected premises. All disease spread was considered lateral spread between infected and susceptible farms. Simulations varied in the availability of resources for conducting disease control activities, including outreach, quarantine, euthanasia/depopulation of detected premises, movement controls, tracing, and active surveillance. We assumed a completely naïve population, and the variable levels of vaccination possibly applied within backyard farms was not modeled explicitly.

Four levels of response were evaluated. A specific combination of integrated control strategies was associated with each response option, with a general increase in response intensity from response 1 to 4 (see Appendix B for detailed information on specific activities modeled in each response option). Disease control activities are identical for the first 161 days of each scenario to reflect the actual outbreak response up to that point in time. Alternative disease control activities, based on resource level, were applied at day 162 of each iteration (75 days post first detection). The model was run for 250-iterations for each of the four response options.

A summary comparison of response options and associated control activities are described in Table 10. The 'X's are provided to estimate a qualitative comparison between control strategies. They are not intended to approximate a quantitative comparison between response options.

Table 10. Qualitative summary comparison of the four alternative response options examined.

Response Option	Surveillance Capacity	Surveillance Zones	Movement Restrictions	Depopulation Capacity	Depopulation Zones
1	X	X	X	X	X
2	XX	X	XX	XX	X
3	XX	XX	XX	XX	XX
4	XXX	XX	XXX	XXX	XXX

Results

The best response option was dependent on the desired outcome. If limiting disease spread, as expressed by the mean number of infected backyard premises, was the only goal, response option 4 achieved the greatest reduction in the number of infected backyard premises. Reducing the total number of infected commercial premises was best achieved with response options 3 or 4.

Both response options 3 and 4 reduced the likelihood of extremely large outbreaks. However, any increase over response option 1 reduced the mean number of infected backyard premises, suggesting that minimal response is unlikely to achieve an adequate reduction in disease spread among backyard premises.

Outbreak duration, as expressed by the percentage of simulated outbreaks continuing into the months following the application of the response option was shortest, on average, for response options 3 or 4. Pronounced differences were observed when comparing response options 1 or 2 with response options 3 or 4, with little difference observed between response options 3 and 4.

Surveillance effectiveness, as expressed by the percentage of infected premises that were detected through passive and active surveillance activities, was significantly improved under response option 4, in comparison with any of the other response options. Little difference was observed in detection rates between response options 1 and 2, with some improvement observed with response option 3.

With all response options, the predominant site of disease spread shifts from San Bernardino County to Los Angeles County shortly after applying the alternative response. This shift was most pronounced with response options 3 or 4. Surveillance surges within the first 30 days post-implementation of the response option generally increased the rates of detection in San Bernardino County and reduced further spread within the county, to the extent that the majority of future infections occurred in Los Angeles County.

The largest and longest simulated outbreaks frequently involve significant disease spread within Los Angeles County, irrespective of the selected response option. A relatively small number of simulated outbreaks became extremely large (greater than 1,000 infections) and persistent (remaining active for at least 3 months following the selection of a response strategy), irrespective of the selected response option.

C. Estimating Within-Flock Transmission Parameters and Predicting the Time to Detect vND in Unvaccinated Flocks

Within-flock disease transmission models are used to evaluate surveillance options, support risk assessments, and assess different control measures. Statistical distributions for bird-level disease state durations are key inputs for within-flock disease transmission models. We estimated bird-level disease state durations and a lower bound on the rate of transmission (β) in unvaccinated flocks using experimental data available from the peer-reviewed literature and unpublished data.

We used the estimated parameters to predict the time to detect vND in an unvaccinated, 50-bird flock, based on observation of increased mortality (2 or more dead birds within a 3-day period).

Methods

Estimating the latent and infectious periods and time to death at the bird level

For this analysis, we defined the latent period as the interval between when an individual bird is exposed to the virus and when it begins shedding virus in detectable concentrations. We estimated the distribution of the latent period from viral shedding data collected on various days post inoculation (DPI), as reported in experimental studies in the literature and from unpublished SEPRL data¹³. Data were available from 122 unvaccinated chickens. Oropharyngeal swabs were collected at specific sampling times post inoculation and starting on 1 or 2 DPI. These data points represented the CA 2018 vND strain, CA 2002-2003 vND strain, and a mesogenic vND strain. An additional 73 data points (birds) were available for the time to death post inoculation (observed at daily intervals). These data included unvaccinated chickens inoculated with vND-CA 2002-2003, vND-CA 2018, vND-Peru 2008 or vND-India 2012. Contact bird data from unvaccinated birds were not included for estimating the infectious period, as data was only available for five birds, and the first sampling time was 2 days post contact. The non-inoculated birds in this experiment all died by day 6 post contact, indicating that the range of time to death is comparable to that for inoculated birds.

The infectious period was defined as the interval from when an individual bird begins shedding virus in detectable quantities to when it either recovers or dies. In several experimental studies, only the time to death was observed, and oropharyngeal swabs were not collected. We jointly fit the parameters of the latent and infectious periods given all the observed data, including instances in which only the time to death was observed.

We used the Metropolis MCMC algorithm implemented in R for parameter estimation. The three chains were run for 10,000 iterations with burn-in of 2,000 iterations. There was no significant autocorrelation beyond 60 lags. Uniform priors with wide limits that included the MLE estimate were used in the current analysis.

Estimating the rate of transmission (β)

The adequate contact rate is a key parameter that determines the rate of within-flock spread. In the Susceptible-Exposed-Infected-Recovered (SEIR) model, the adequate contact rate or the transmission parameter (β) is the average number of contacts that a bird has with other birds per unit time, such that

¹³ Courtesy of Kiril M. Dimitrov, Helena L. Ferreira, Mary Pantin-Jackwood, Tonya L. Taylor, Iryna V. Goraichuk, Claudio L. Afonso, David L. Suarez

the contact can transmit infection. We estimated the adequate contact rate using data provided in Miller et al., 2003, in which the transmission to contact birds was studied. We used direct forward simulation to obtain the posterior distribution for the adequate contact rate, given the observed experimental data on viral shedding and the timing of death for the contact birds.

Estimating Time to Detection in Unvaccinated Flocks

We estimated the time to detection in flocks of 50 unvaccinated birds using a stochastic within-flock simulation model (SEIR), applying the maximum likelihood estimates of the parameters (as described above) and a trigger criterion of observing 2 or more dead birds within a 3-day period.

Results

Latent and infectious periods and time to death at the bird level

- Latent Period Parameters

The mean latent period was 0.40 days (95 percent CI: 0.30 – 0.51 days).

- Infectious Period

The mean infectious period was 4.33 days (95 percent CI: 4.03-4.98 days). The maximum likelihood estimate for the infectious period was shape parameter of 13.07 (95 percent CI: 3.6-18.6) and a scale of 0.33196 (95 percent CI: 0.23-1.33).

- Time to Death

The maximum likelihood estimate for the bird-level mean time to death was 4.73 days (95 percent CI: 4.45-5.4 days).

Rate of transmission (β)

There was considerable uncertainty for this parameter, given the limited amount of data available. However, based on the estimated posterior, a value of 1.7 contacts per day (95 percent CI: 1.69-9.79 adequate contacts per day) may be used as a conservative estimate.

Time to detection in unvaccinated flocks

Under the baseline scenario, the time to detection was 5.5 days (95 percent PI: 4-7 days) based on 20,000 iterations of the model.

D. Predicted Disease Mortality and Infection Prevalence in vNDV Infected Flocks Using a Disease Transmission Simulation Model

We used a stochastic within-flock vND transmission model to predict the prevalence of infectious birds and cumulative mortality over time in infected flocks (a flock in this analysis was defined as birds in a house or barn). The model results provide a general idea about the possible time elapsed since the onset of infectiousness based on the observed cumulative mortality levels in the flock.

We evaluated model scenarios for unvaccinated or vaccinated commercial and backyard flocks. Results for vaccinated commercial flocks (20,000 birds) and both vaccinated and unvaccinated backyard flocks (400 birds) are provided in the main text. The results for additional flock sizes and vaccination scenarios are included in Appendix C.

Overall, the model results indicated low levels of mortality in vaccinated flocks due to a greater fraction of recovering birds; cumulative percent mortality over 4.5-5.0 percent was rarely noted in modeled outcomes for these flocks. Furthermore, disease spread was predicted to be slower in vaccinated flocks compared to unvaccinated flocks. Disease spread in unvaccinated flocks was predicted to be fairly rapid with extensive disease mortality (i.e. 50 percent mortality after 7-12 days of infectiousness in a backyard flock with 400 birds). The results also indicated that the time to attain specific percent cumulative mortality levels is longer for larger flocks. We note that the model results are approximate as there is considerable uncertainty in key parameters such as the adequate contact rate and the vaccine efficacy under field conditions.

Methods

We used a stochastic individual based transmission model to simulate vND spread in commercial and backyard flocks. The model simulates the number of birds in susceptible, latent, infectious and recovered or dead states in 0.01-day time steps. The model allowed for a fraction of the birds in a vaccinated flock to be immune. The predicted mortality and prevalence curves were based on 10,000 model iterations.

Model parameters for unvaccinated flock scenarios were estimated from available experimental inoculation studies for the CA/2018 and other vND strains as described in Section V: Part C. (Dimitrov, 2019 #3). The durations of latent and infectious periods for vaccinated flocks were estimated from experimental data presented in Miller et al. (2013) as described in Appendix C. The adequate contact rate and disease mortality in vaccinated flocks were based on estimates from outbreak data from commercial flocks as described in the Section V: Part E. Additional details of the model parameters are provided in Appendix C. Predicted Disease Mortality and Infection Prevalence Under Additional Flock Size And Vaccination Scenarios.

Results

Vaccinated commercial flocks

The model results on the cumulative mortality percent¹⁴ and the prevalence of infectious birds on various days post infection for a vaccinated commercial flock of size 20,000 birds are provided in Figure 11. The observed cumulative mortality percent at a time point can provide an approximate indication of the number of days post onset of infectiousness in the flock.

¹⁴ Defined as the cumulative number of dead birds divided by the beginning flock size multiplied by 100.

Table 11 provides the predicted days post the onset of infectiousness when various cumulative mortality levels were attained. For example, in the 20,000-bird vaccinated commercial flock, it took 17.4 (90 percent P.I. 13.8-22.0) days after the onset of infectiousness for the cumulative mortality to reach 2 percent of the starting flock size. In this analysis, the time to onset of infectiousness was defined as the earliest time point when one or more birds were infectious. Cumulative mortality in vaccinated commercial flocks remained relatively low. Cumulative mortality of at least 4.5 percent of the flock was observed in only 2.07 percent of the simulation iterations. Predicted daily mortality is shown in Figure 12, which was predicted to peak about 17 days post exposure.

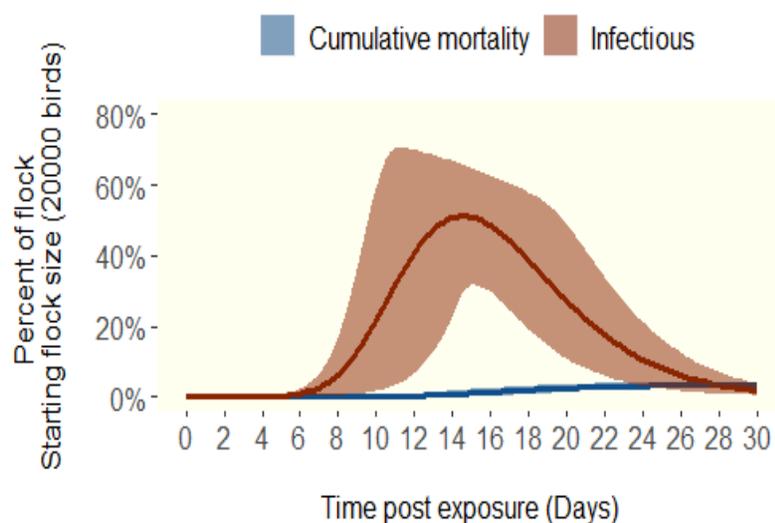


Figure 11. Predicted cumulative mortality percent and prevalence of infectious birds on various days post exposure in a 20,000 vaccinated commercial cage free layer flock using baseline vND spread parameters. Shaded area represents the 90 percent prediction interval for each variable.

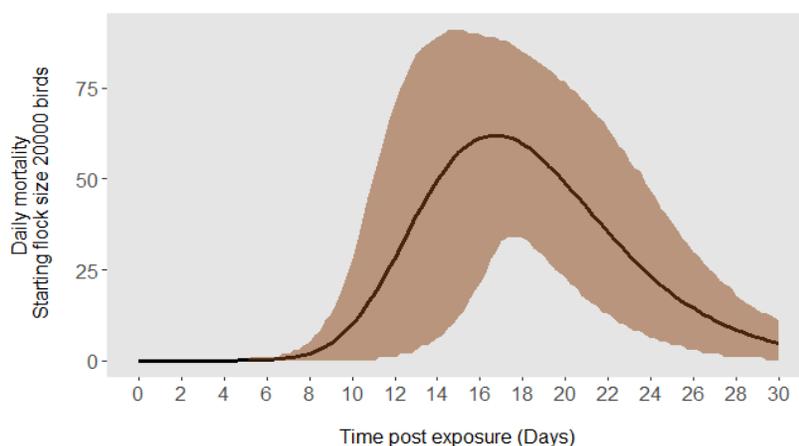


Figure 12. Predicted daily mortality on various days post exposure in a 20,000-bird vaccinated commercial cage free layer flock using baseline vND spread parameters. Shaded area represents the 90 percent prediction interval.

Table 11. Predicted days post onset of infectiousness to attain various cumulative mortality levels in a 20,000-bird vaccinated commercial cage free layer flock using baseline vND spread parameters.

Cumulative Percent Mortality	Percent of simulation iterations in which this cumulative mortality is attained	Predicted number of days post onset of infectiousness to reach this cumulative mortality percent. median (90% Prediction Interval)
2	95.77	17.4 (13.8-22)
2.5	95.68	19.1 (15-24.2)
3	84.18	21.1 (16.5-27)
3.5	54.3	22.5 (17.8-28)
4	24.13	24.4 (19.8-28.8)
4.5	2.07	26.4 (22.5-29.5)
5	0	NA

Results for Unvaccinated backyard flocks

Model results on the cumulative mortality percent and the prevalence of infectious birds on various days post infection for an unvaccinated backyard flock (400 birds) are provided in Figure 13. Table 12 provides the predicted days post the onset of infectiousness when various cumulative mortality levels were attained, and the predicted daily mortality is shown in Figure 14. Based on these results, unvaccinated backyard flocks are predicted to have rapid spread of disease with high levels of mortality.

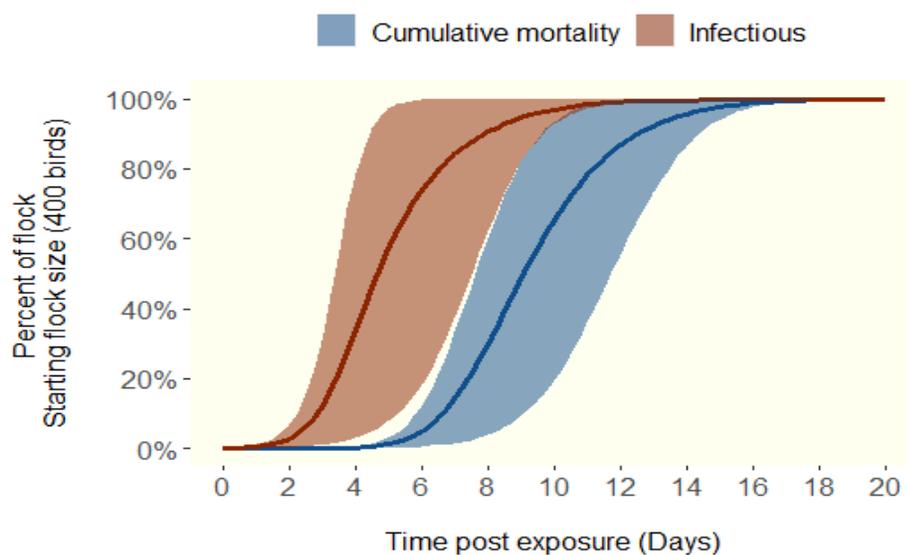


Figure 13. Predicted cumulative mortality percent and prevalence of infectious birds on various days post exposure in a 400-bird unvaccinated backyard flock using baseline vND spread parameters. Shaded area represents the 90% prediction interval for each variable.

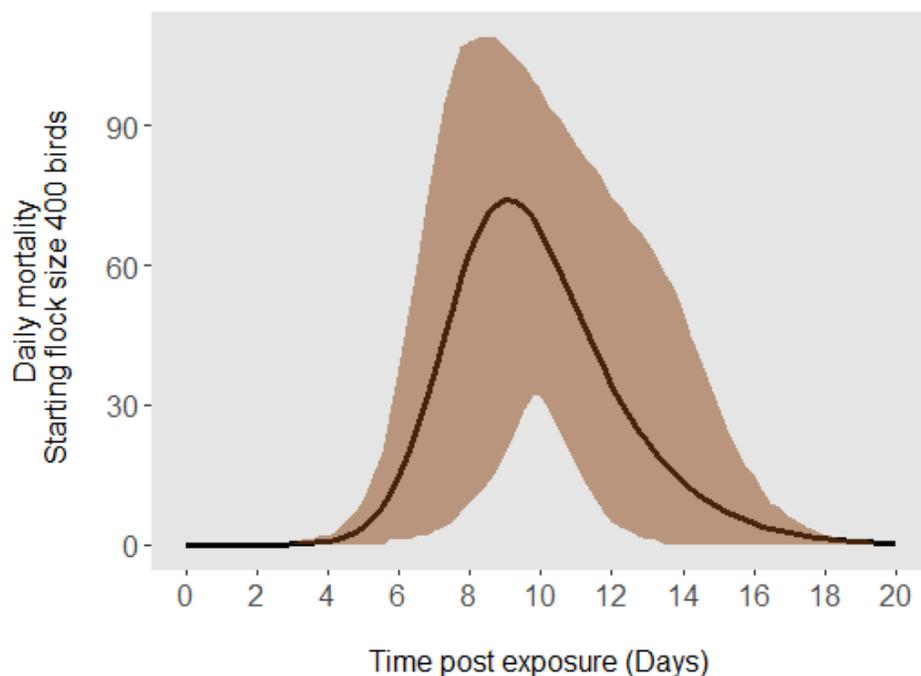


Figure 14. Predicted daily mortality on various days post exposure in a 400-bird unvaccinated backyard flock using baseline vND spread parameters. Shaded area represents the 90% prediction interval.

Table 12. Predicted days post onset of infectiousness to reach various cumulative mortality levels in a 400-bird unvaccinated backyard flock using baseline vND spread parameters.

Cumulative Percent Mortality	Predicted number of days post onset of infectiousness to reach this cumulative mortality percent. median (90% Prediction Interval)
10	6.8 (5.2-9.5)
20	7.6 (6-10.5)
30	8.1 (6.5-11)
40	8.5 (6.8-11.8)
50	8.9 (7.2-12.2)
60	9.4 (7.5-12.8)
70	9.8 (8-13.2)
80	10.4 (8.5-14)
90	11.1 (9-15)

Results for Vaccinated Backyard Flocks

Model results on the cumulative mortality percent and the prevalence of infectious birds in vaccinated backyard birds on various days post infection are provided in Figure 15. The predicted days post the onset of infectiousness when various cumulative mortality levels were attained are provided in Table 13, and the predicted daily mortality is provided in Figure 16. As would be expected, disease spread is predicted to be slower in vaccinated backyard flocks with lower mortality, as compared to the results predicted for unvaccinated backyard flocks. The predicted time to reach certain cumulative mortality levels in the vaccinated backyard flocks was shorter than vaccinated commercial flocks, which are typically much larger.

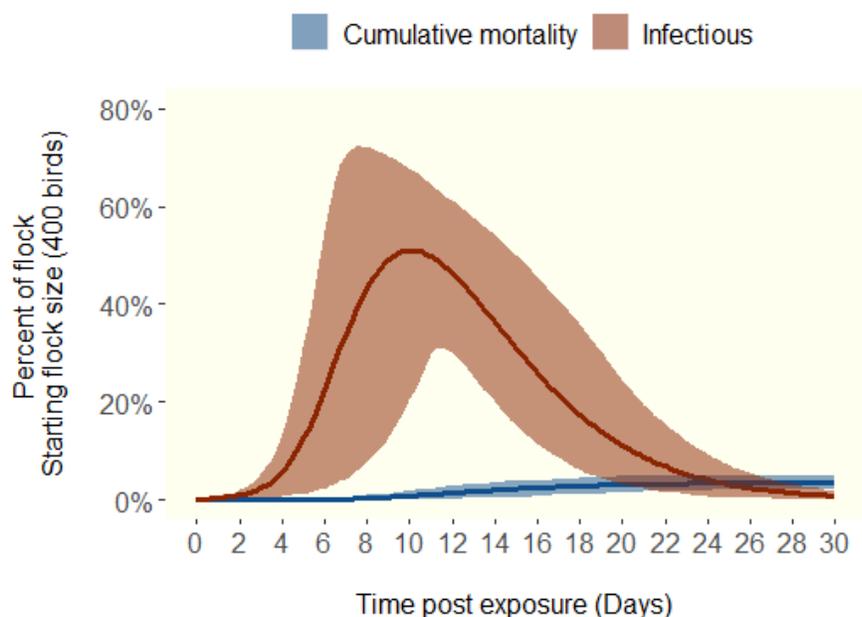


Figure 15. Predicted cumulative mortality percent and prevalence of infectious birds on various days post vND exposure in a 400-bird vaccinated backyard flock. Shaded area represents the 90% prediction interval for each variable.

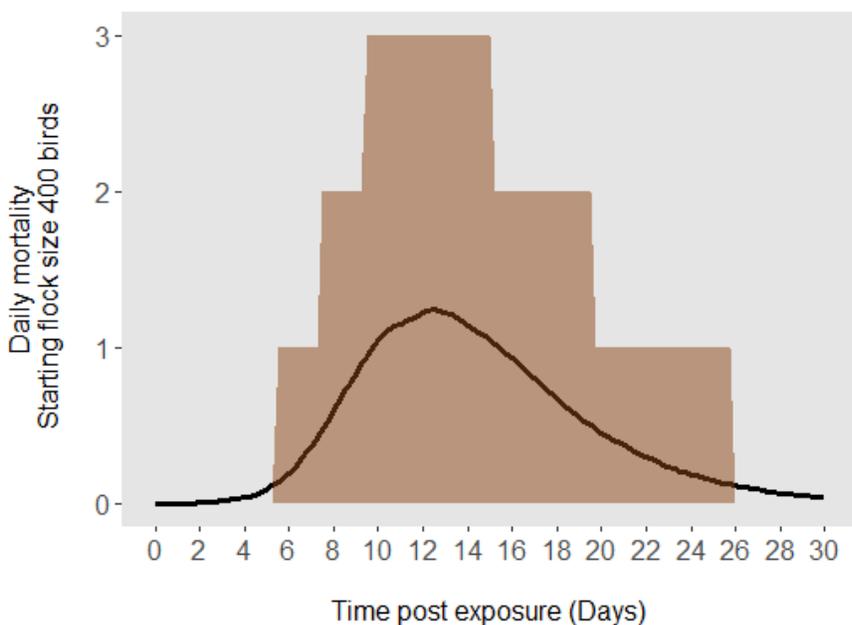


Figure 16. Predicted daily mortality on various days post vND exposure in a 400-bird vaccinated backyard flock. Shaded area represents the 90% prediction interval.

Table 13. Predicted days post onset of infectiousness to reach various cumulative mortality levels in a 400-bird vaccinated backyard flock using baseline vND spread parameters.

Cumulative Percent Mortality	Percent of simulation iterations in which this cumulative mortality is reached	Predicted number of days post onset of infectiousness to reach this cumulative mortality percent. Median (90% Prediction Interval)
2	91.23	13.5 (9-20.2)
2.5	84.72	14.8 (9.8-22.2)
3	73.26	16.1 (10.5-23.8)
3.5	57.17	17.1 (11.2-25.2)
4	39.57	18 (12.2-25.8)
4.5	23.53	18.8 (12.8-26.8)
5	12.64	19.5 (13.8-27.2)

Conclusions

We predicted the prevalence of infectious birds and the cumulative mortality percent in unvaccinated and vaccinated flocks using a stochastic within flock transmission model. The model results indicate a

fairly rapid disease spread in unvaccinated flocks with extensive disease mortality. The predicted time to attain specific percent cumulative mortality levels was longer for the larger commercial flocks. The transmission dynamics in vaccinated flocks were markedly different from those for unvaccinated flocks with a slower disease spread and lower disease mortality. We note that the model predictions are approximate and should be used cautiously as there is a significant uncertainty and variability in key parameters such as the adequate contact rate and vaccine efficacy in backyard and commercial flocks.

E. Estimating the Time of Disease Introduction in vNDv Infected Commercial Layer Barns Using Egg Production and Mortality Data

Determining the time of vND virus introduction in a flock is an important part of outbreak investigations. By narrowing the time window of possible virus introduction, we can better identify the potential routes of the virus introduction and enhance our understanding of the pattern of disease spread. In this analysis, egg production, diagnostic testing and daily mortality data were used to estimate the most likely date of virus introduction for four confirmed vNDv infected barns with vaccinated birds in a commercial layer premises in California.

Summary

The analysis was performed using a within-house disease transmission model along with approximate Bayesian computation (ABC) to estimate the distribution of the times of exposure that resulted in a smaller difference between the model predictions and the observed production and diagnostic testing data. Approximate Bayesian computation algorithms as described in Marjoram *et al.*, 2003 were used to estimate the time of virus introduction into the barn, the adequate contact rate and other model parameters.

The estimated time of introduction ranged from 28 November 2018 (95 percent C.I., 6 November - 10 December 2018) for barn D to 25 December 2018 (95 percent C.I., 17 December -26 December 2018) for barn A. The adequate contact rate (a parameter that impacts the rate of disease transmission in a barn) was the highest in barn A with 1.42 (1.02-4.72) contacts per day for 102-week-old cage free birds and the lowest in barn D with 0.33 (0.26-0.58) contacts per day for 33 week old caged layers. The ABC estimation procedure is also useful to inform other model parameters such as the likelihood that an infected bird dies due to vND or the egg production rate among infected birds. The estimated parameters are beneficial in models used to inform risk analysis, surveillance design and developing scenarios for emergency preparedness exercises.

The results of this analysis are subject to considerable uncertainty due in part to a limited number of experimental studies with vaccinated layer birds, and uncertainties associated with vaccine efficacy in commercial flocks of different ages and breeds. Nevertheless, the results demonstrate the usefulness of production and testing data to understand the transmission dynamics of vND under field conditions.

Methods

Summary of production and diagnostic data

The analysis was performed for four barns confirmed as vNDv infected in a commercial layer premises in California. The operation had a total of 21 occupied barns. Three barns (barns A, B, and C) housed cage-free laying hens, while barn D had caged layers. All 4 barns were positive for vNDv based on RRT-PCR

testing of dead bird samples collected on 3 January 2019. Five dead birds per barn were tested in each of the 4 positive barns on this sampling date. Barns B and C were also sampled on 17 December 2018 and tested negative via RRT-PCR.

Barn level daily egg production data were provided for 12 days beginning 24 December 2018. The egg production drop was quite variable among different barns. A greater than 40 percent drop in egg production was observed in barn A, which housed birds of age 102 weeks, while a very mild drop in egg production was observed in the other barns, which housed birds of age 131 or 33 weeks. Approximately 4 months of daily mortality data before detection were provided for barns A, B and C, while 12 days of daily mortality data were provided for barn D. Similar to the egg production, the daily mortality was also quite variable with doubling of mortality in some of the infected barns and milder elevations above baseline in others.

Overview of modeling approach

We used ABC to estimate the likely time of virus introduction and the key model parameters such as the adequate contact rate (a parameter which regulates the rate of within flock disease spread) and the fraction of infected birds that die from the available production and test data.

A stochastic individual based simulation model was first used to simulate the disease mortality, infection prevalence over time and egg production rate for a wide range of model parameters such as the adequate contact rate, times of disease introduction and disease mortality (i.e. prior distributions). In the next step, the sum of squared distance between the model predicted daily mortality and egg production and the observed data, and the difference between observed and simulated diagnostic test results was calculated as a measure of deviation between the model output and data (ψ). The ABC algorithm was then applied to simulate the model under input values. The parameters in model iterations where the metric ψ was sufficiently small, indicating a good fit to the data, were then accepted to estimate the distribution of the time of introduction and other model parameters.

The transmission model parameters for vaccinated flocks were estimated from current outbreak data and experimental data presented in Miller et al. (2013) from vaccinated SPF chickens and contact birds. The mean latent period was 0.39 days while the mean infectious period in vaccinated birds was 4.6 days. Implementation details of the ABC procedure are provided in Appendix D.

Finally, the modeling methods were also validated by estimating the time of introduction using a grid-based simulation and Euclidian distance-based likelihood approach which gave comparable time of introduction estimates to the ABC procedure for barn A.

Results

Of the four barns included in the analysis, results for barn A produced the lowest uncertainty due to a marked increase in mortality and drop in egg production beyond the normal production range for that barn. The model fits of the egg production rate and daily mortality for barn A are shown in Figure 17 and Figure 18, respectively. Model fits for the other barns are provided in Appendix D. We observe that estimated egg production and daily mortality from the model closely matches the data, indicating a reasonable fit. The posterior distribution for the adequate contact rate and time of introduction for barn A is shown in Figure 19 and Figure 20, respectively.

The results for the estimated time of introduction and the adequate contact rate are summarized in Table 14. From Table 14, barn D, which housed caged layers, had the earliest date of introduction with a slower contact rate, while barn A had the latest estimated day of introduction. However, the intervals for the estimated day of introduction are overlapping for barns A, B and C.

The results also indicate that the disease mortality in the vaccinated commercial flocks can be relatively low (2.7 percent, 95 percent C.I., 1.5 percent-4.7 percent for barn A) compared to the estimates from experimental studies (11 percent) (Miller, 2013). The drop in egg production due to vNDv infection was likely lower in barns B, C and D relative to barn A as model parameters representing smaller drops in egg production due to vNDv infection resulted in a better fit for these barns. The median egg production rate in infected birds from the model results was quite variable, for example 55 percent for barn C and 24 percent for barn A.

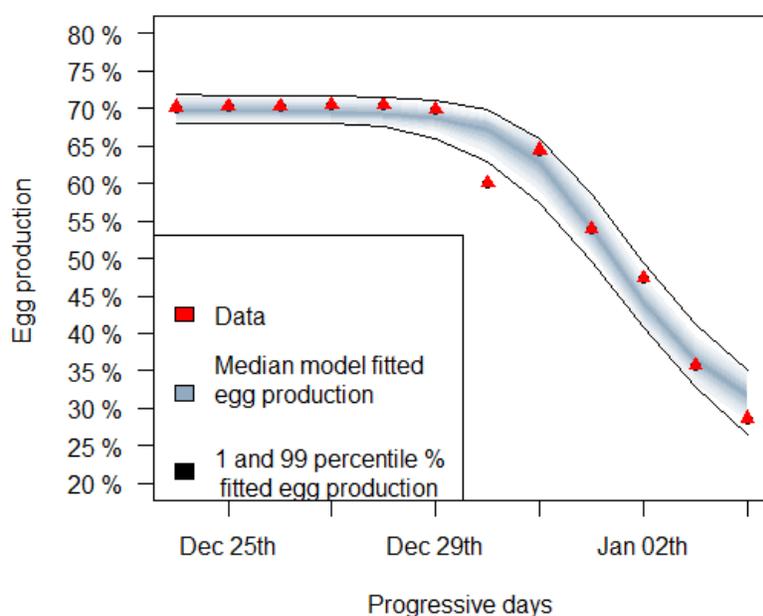


Figure 17. Model-fitted egg production rate curves from the approximate Bayesian computation and observed egg production rate for Barn A. The shaded region represents the 95 percent credibility interval for the fitted egg production.

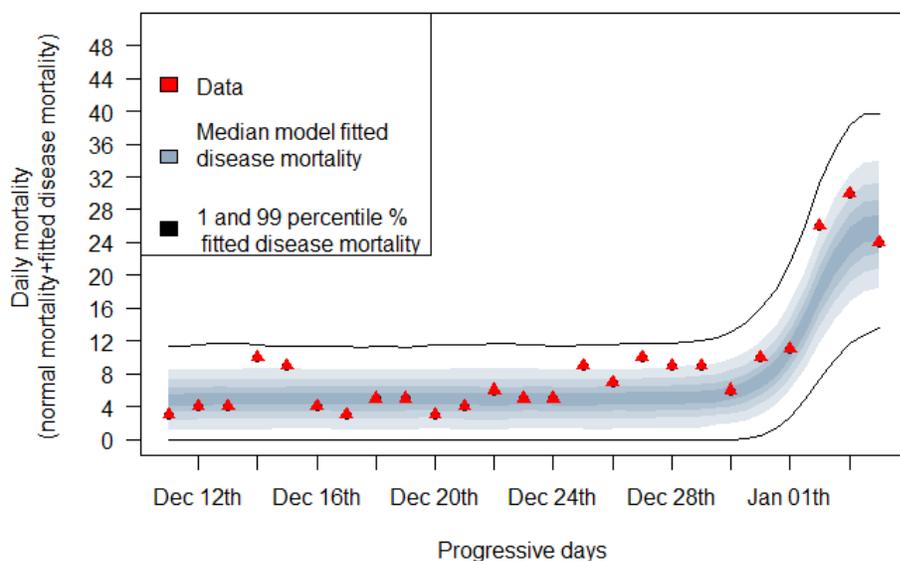


Figure 18. Model fitted disease plus normal mortality from the approximate Bayesian computation and the observed daily mortality for Barn A. The shaded region represents the 95 percent credibility interval for the fitted daily mortality.

Table 14. Estimated time of introduction and adequate contact rate for each of the four barns.

Barn	Estimated mode, median, (95% C.I.) of time of introduction	Estimated mode, median, (95% C.I.) of adequate contact rate (contacts per day)
A (cage free layers)	12/25/2018, 12/23/2018 (12/17/2018 -12/26/2018)	1.42, 1.86 (1.02-4.72)
B (cage free layers)	12/8/2018, 12/6/2018 (11/17/2018 -12/17/2018)	0.48, 0.53 (0.36-0.84)
C (cage free layers)	12/9/2018, 12/7/2018 (11/24/2018 -12/20/2018)	0.5, 0.68 (0.34-2.35)
D (caged layers)*	11/28/2018, 11/24/2018 (11/6/2018 -12/10/2018)	0.33, 0.36 (0.26-0.58)

*Results from barn D should be interpreted cautiously as the drop in egg production was mild to non-existent, increasing the uncertainty in the estimated results.

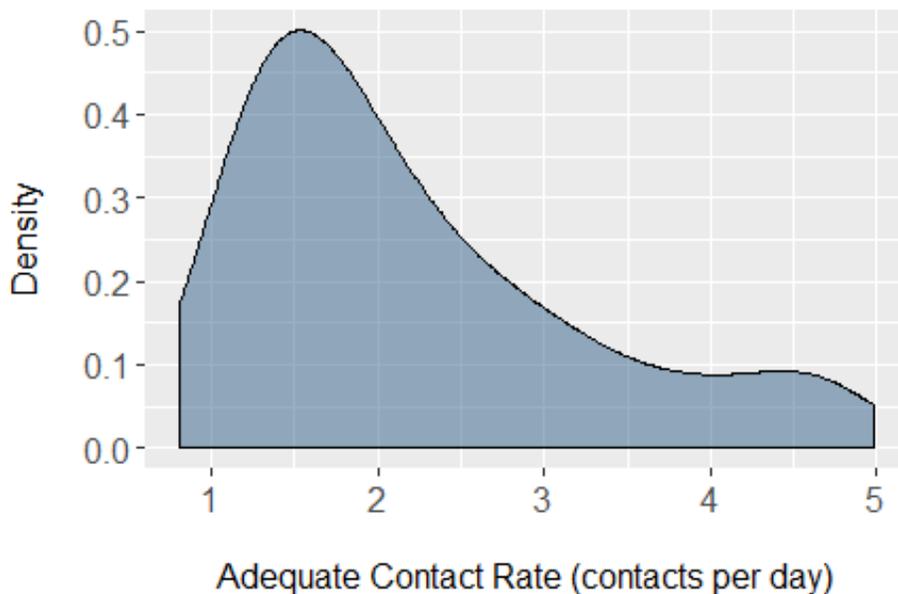


Figure 19. Posterior distribution for the adequate contact rate for Barn A.

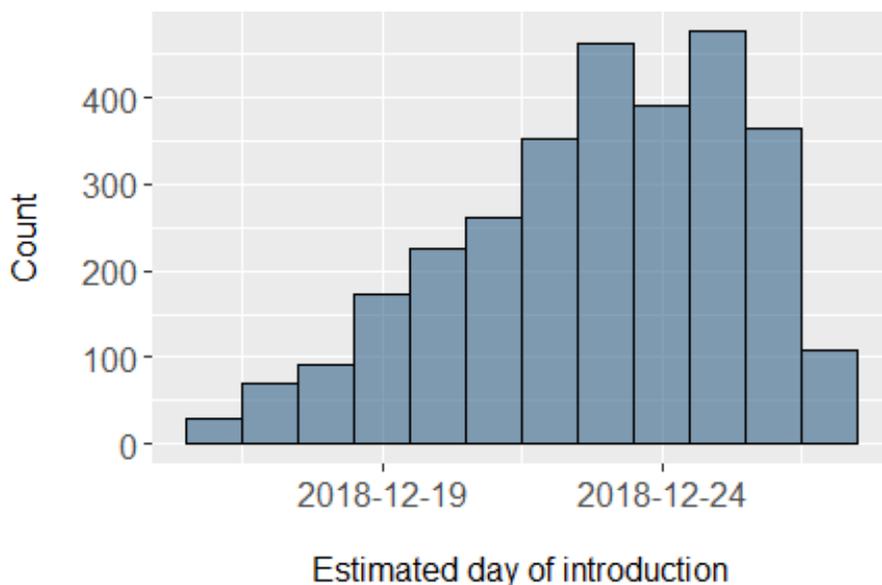


Figure 20. Histogram of the Posterior distribution for the time of vND introduction for Barn A.

Discussion

Flock daily mortality, egg production rate and available diagnostic test data can be used to estimate the time of virus introduction in a vND infected barn. By narrowing the time window of possible virus introduction, we can better identify the potential routes of virus introduction and enhance our understanding of the pattern of disease spread. We estimated the time of introduction for four

confirmed vND infected barns in a commercial layer premises in California using a stochastic simulation model together with approximate Bayesian computation (ABC). Approximate Bayesian computation is suitable for parameter estimation when explicit calculation of the likelihood is intractable. The ABC approach has been used to estimate the time of disease introduction using field mortality data for other diseases such as ASF (Guinat, 2018).

As can be observed in Table 14, the estimated likely time of vNDv introduction for barn A ranged from 12/17/2018 -12/26/2018, 13-4 days before observing a considerable drop in egg production rate. Barn A was a cage free layer house with older birds (102 weeks) and showed a more than 40 percent drop in egg production over a two-week period along with increased mortality. The estimated adequate contact rate was the highest for this barn, indicating a faster rate of disease spread.

Barn D, which housed 33-week-old caged layers, had the earliest estimated date of vNDv introduction of 11/28/2018 (95 percent C.I., 11/6/2018, 12/10/2018). The estimated contact rate was lower for this barn, which could possibly be due to housing younger birds with a greater immunity or slower disease transmission among birds housed in cages. However, the results for this barn need to be interpreted cautiously as the drop in egg production was very mild resulting in a greater uncertainty in the estimates. Additional daily mortality and egg production data may help obtain more precise time of introduction estimates for this barn.

The fall in egg production and increase in daily mortality were also fairly mild in barn B and barn C, leading to greater uncertainty in the estimate for the time of virus introduction as compared to the estimate for barn A. Lower contact rates, as were estimated for barns B and C, can also lead to greater uncertainty in the time of virus introduction estimates. In general, a significant deviation in the production parameters from baseline in the field data is required to estimate the time of virus introduction.

The results suggest spread of vND in vaccinated commercial barns can be relatively slow (adequate contact rate estimate was less than 1 for barns B, C, and D). This can have important implications for surveillance design as slow spread can lead to a less pronounced presence of clinical signs in the flock, for example. The ABC estimation procedure is also useful to inform other model parameters such as the likelihood that an infected bird dies due to vND or impact on the egg production rate among infected birds. Our results indicated that the disease mortality in the vaccinated commercial flocks can be relatively low (2.7 percent, 95 percent C.I. 1.5 percent-4.7 percent based on barn A). The estimated egg production rate among diseased birds varied markedly between different barns. For example, the median estimated egg production rate in vNDv infected birds was 24 percent for barn A and 55 percent for barn C. The adequate contact rate, disease mortality parameter and other parameter estimates are beneficial to inform risk assessment and active surveillance models and for developing scenarios in emergency preparedness tabletop exercises.

There is considerable uncertainty in the estimated time of virus introduction and adequate contact rate due to limited data on key model input parameters such as level of immunity among vaccinated flocks of different ages and breeds, disease mortality in vaccinated birds, and the mild drop in egg production and mild elevation in mortality in some of the barns. Nonetheless, the analysis presented here demonstrates the value of production data and diagnostic testing data and its ability to provide information on disease dynamics within a poultry flock.

F. Spatial and Spatiotemporal Patterns of the Outbreak

Knowledge of disease patterns in space and time can identify areas at higher risk for disease spread and allow disease control, prevention, and surveillance strategies to be implemented effectively (Ward, 2007). We performed a spatiotemporal analysis on confirmed and presumptive positive vND in backyard/exhibition premises in California. We obtained data on confirmed and presumptive positive premises from in-person interviews using the CDFA Non-Commercial Premises Virulent Newcastle Disease Epidemiology questionnaire, which were entered into the USDA's Emergency Management Response System (EMRS). For population data, we used the results of a spatial analysis predicting the geographic area and density of backyard bird ownership in California at the census block level (see Section III, Part A: Predicting Areas of Backyard Bird Ownership).

Methods

We used spatial and spatiotemporal scan statistics to detect statistically significant high-risk clustering of vND cases (Kulldorff, 1997). For the analysis, we defined cases as confirmed or presumptive positive premises. Data from 435 cases detected from 16 May 2018 to 22 February 2020 located within the California Regional Quarantine Area (RQA) in Los Angeles, Riverside, and San Bernardino Counties were included in the analysis. Case information consisted of the location, reported date of onset of clinical disease, and presumptive and confirmed positive diagnosis date. Based on the phylogenetic analysis (see Section II, Part A: Phylogenetic Analysis and Diagnostics), spatial and spatiotemporal analyses were performed separately based on the two main vNDV sub-groups (vNDV-01 and vNDV-02) since premises within the same sub-group are genetically related, and premises between sub-groups are genetically unrelated. Sub-groups vNDV-01 and vNDV-02 contained a total of 147 and 288 premises, respectively. Cases were aggregated at the census block level within each county. Population information consisted of the estimated number of premises of predicted backyard bird ownership in each census block for the outbreak area. We used the centroid (latitude, longitude) of each census block as location information for the analysis.

A Poisson model was used to estimate the number of cases that might be expected to occur in the absence of any clustering. For both the spatial and spatiotemporal cluster analyses, data were scanned with a 5-km radius spatial window. For the spatiotemporal cluster analysis, a temporal window of 21 days was used, which is the World Organisation for Animal Health (OIE) reported incubation period for vND (OIE, 2012). We determined statistical significance (p -value < 0.05) of clusters using the likelihood ratio test and 999 iterations of Monte Carlo simulation implemented in SaTScan (version 9.6).

Results

The 435 detected cases from 16 May 2018 to 22 February 2020 were located within 26 control areas. The cases were located within 93 census blocks, with the number of cases ranging from 1 to 48 premises within an individual block. Riverside County had the highest number of reported cases, with 244 of the 435 detected cases occurring in this area, followed by San Bernardino and Los Angeles Counties (148 and 43 cases, respectively; Figure 21 and Table 15).

The primary or most likely spatial and spatiotemporal statistically significant clusters (p -value < 0.001) of detected vNDV-01 and vNDV-02 cases occurred in Riverside and San Bernardino Counties, respectively (cluster 1 in Figure 22 and Figure 23). In the vNDV-01 primary spatiotemporal cluster, 38 cases occurred

out of an estimated population at risk of 249 backyard/exhibition premises from 3 – 13 December 2018 (Table 15, Figure 24). In this cluster, the relative risk of cases occurring in this area and time period was 1,220.63 times more likely, relative to outside this area. In the vNDV-02 primary spatiotemporal cluster, 30 cases occurred out of an estimated population at risk of 440 backyard/exhibition premises from 18 June – 8 July 2018 (Table 15 and Figure 24). In this cluster, cases were 230.35 times more likely to occur relative to outside this area.

An additional two spatial and five spatiotemporal vNDV-01 clusters, and five spatial and 10 spatiotemporal vNDV-02 clusters, were identified in the three-county area of the RQA (Figure 22 and Figure 23). The number of cases within the spatial clusters ranged from four to 94 cases, whereas the number of cases ranged from three to 38 in the spatiotemporal clusters. The relative risk for vNDV-01 cases was highest in Riverside County, followed by San Bernardino County, whereas the relative risk for vNDV-02 cases was highest in San Bernardino County followed by Riverside County (Table 15). Figure 24 compares the temporal occurrence for vNDV-01 and vNDV-02 spatiotemporal clusters. Most statistically significant spatiotemporal clustering for the entire outbreak occurred during the months between December and March.

Seventy-one of the 435 cases detected during the time period of this analysis did not occur in any spatial or spatiotemporal cluster.

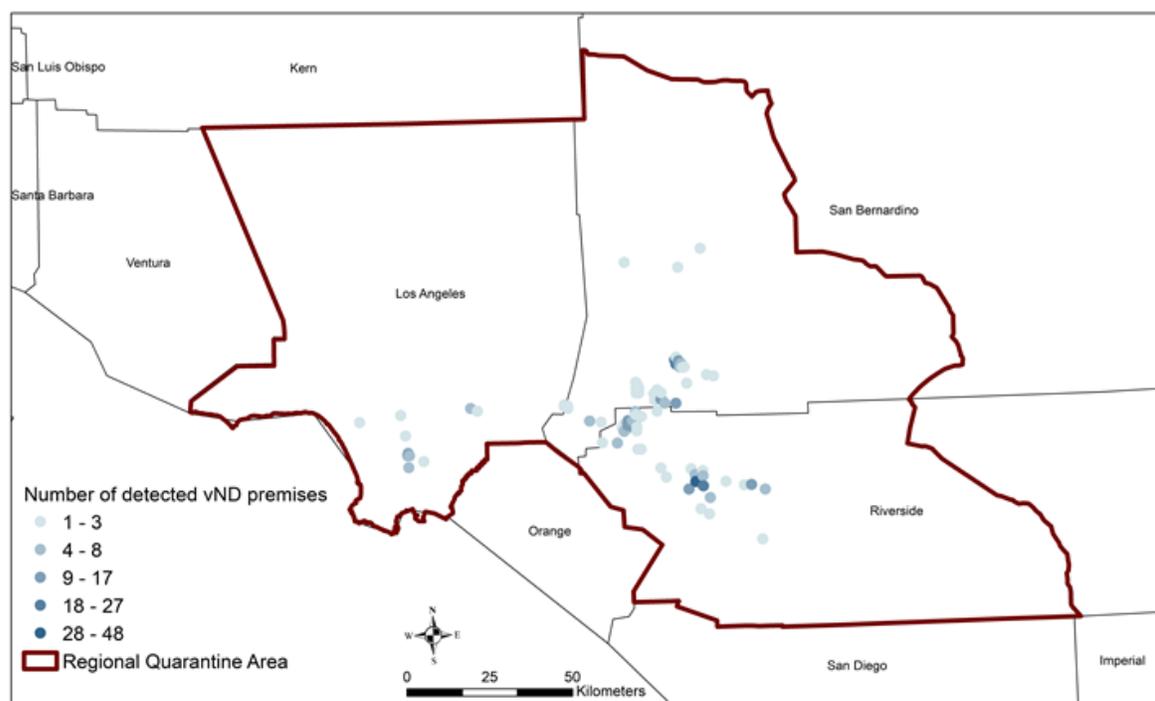


Figure 21. Cumulative number of confirmed and presumptive positive vND premises detected in California from 16 May 2018 to 22 February 2020; data are aggregated at the census block level and the point location of the centroid of each census block is displayed.

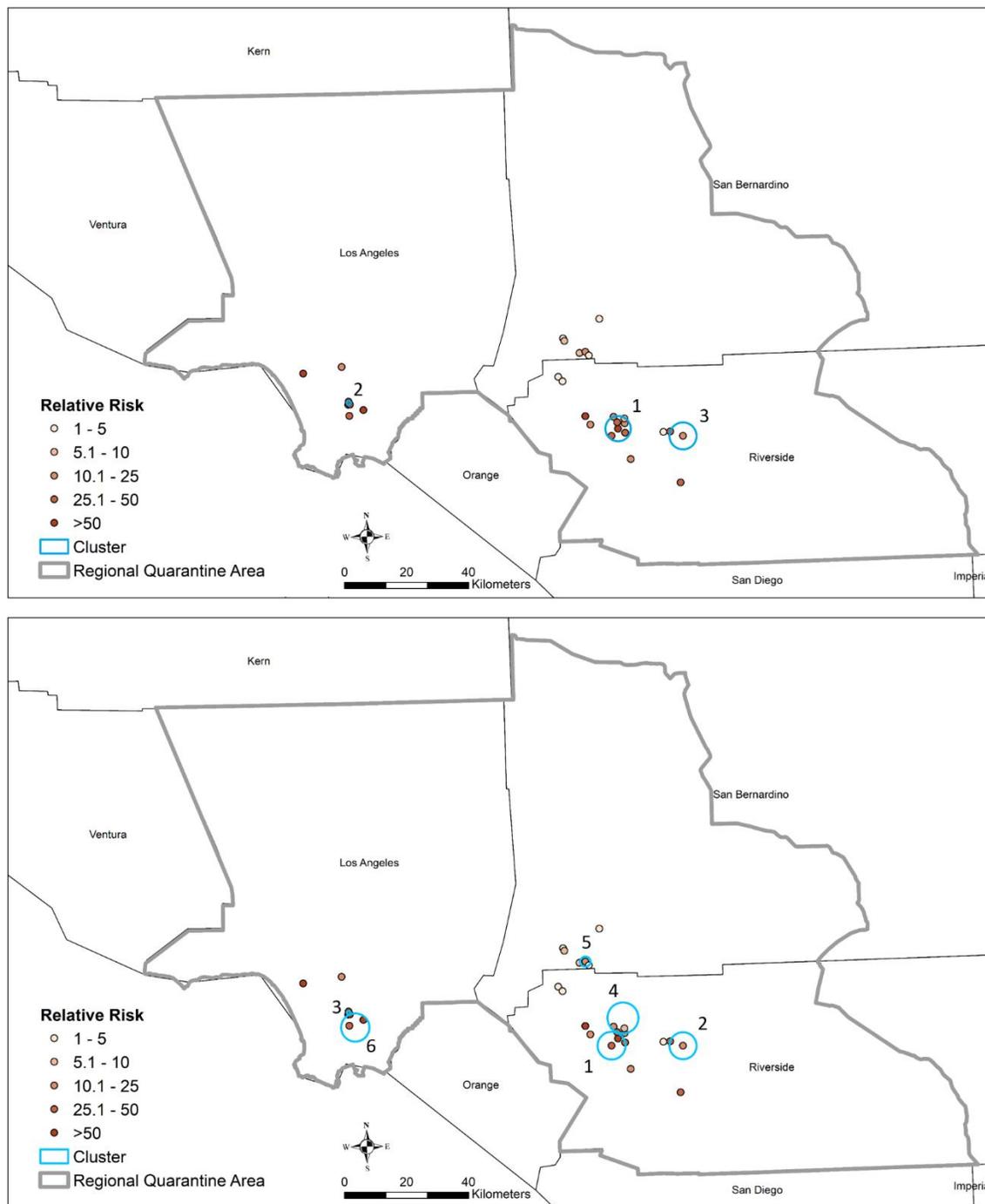


Figure 22. Location of spatial (upper) and spatiotemporal (lower) clusters of vNDV-01 premises and the relative risk of cases occurring within census blocks in California from 16 May to 22 February 2020. Data are aggregated at the census block level and the census block centroids are displayed. Clusters were statistically significant at the $p < 0.05$ level.

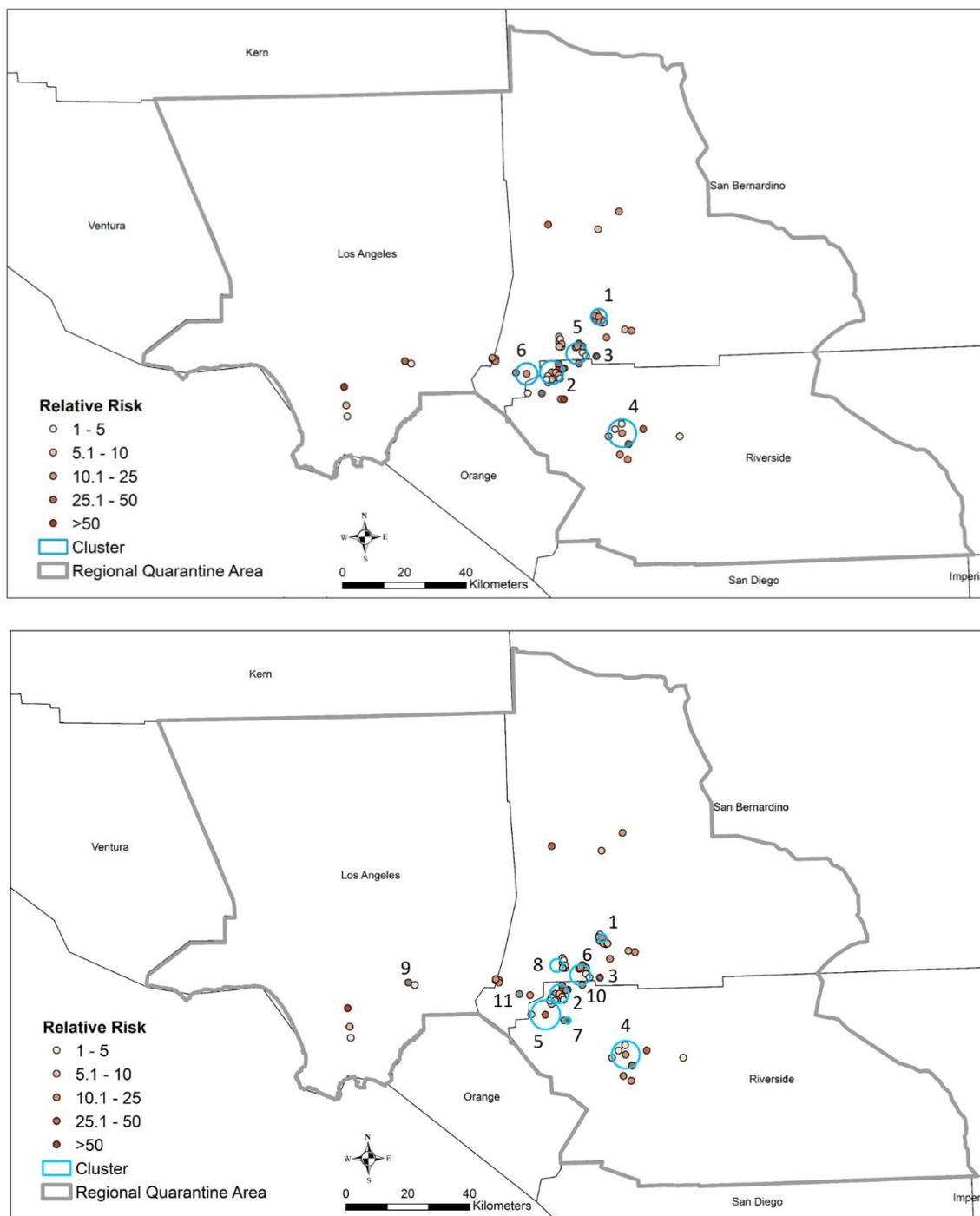


Figure 23. Location of spatial (upper) and spatiotemporal (lower) clusters of vNDV-02 premises and the relative risk of cases occurring within census blocks in California from 16 May to 22 February 2020. Data are aggregated at the census block level and the census block centroids are displayed. Clusters were statistically significant at the $p < 0.05$ level.

Table 15. Spatiotemporal clusters of vND cases in California from 16 May 16 2018 to 22 February 2020.^a

Genetic Sub-group	Cluster ^b	Radius (km)	Time Period	Estimated Population ^c	No. Cases	No. Exp.	Log Likelihood Ratio	Relative Risk	
vNDV-01	Riverside County								
	1	4.49	December 3 – 13, 2018	249	38	0.042	226.15	1,220.63	
	2	4.35	January 19 – February 7, 2019	98	11	0.017	60.95	719.66	
	4	4.98	January 7 – 25, 2019	185	8	0.028	37.43	299.80	
	Los Angeles County								
	3	0.60	July 16 – August 4, 2018	68	9	0.011	51.73	878.14	
	6	4.55	August 22 – September 11, 2018	494	8	0.083	28.82	101.54	
	San Bernardino County								
	5	1.64	May 24 – 31, 2018	182	6	0.012	31.58	535.38	
	vNDV-02	San Bernardino County							
1		1.36	June 18 – July 8, 2018	440	30	0.15	131.65	230.35	
3		0 ^d	December 12 – 23, 2019	10	8	0.0017	59.63	4,756.71	
6		0	January 3 – 7, 2020	2	3	0.00016	26.58	19,277.31	
8		2.10	February 17 – 21, 2019	44	4	0.0035	24.24	1,172.42	
11		0	February 16 – March 7, 2019	24	4	0.0075	21.13	537.35	
Riverside County									
2		2.99	January 7 – 23, 2019	778	22	0.21	81.60	114.44	
4		4.49	January 17 – February 1, 2019	329	13	0.083	53.11	164.41	
5		4.75	February 14 – March 5, 2019	289	8	0.091	28.02	90.50	
7	1.01	January 28 – February 7, 2019	12	4	0.0021	26.28	1,954.05		
10	3.27	December 5 – 18, 2019	268	6	0.059	21.85	103.83		
Los Angeles County									
9	0	July 31 – August 20, 2018	13	4	0.0043	23.38	944.81		

No.: number; Exp.: expected

^aAll clusters were statistically significant (p -value < 0.001)

^b1, primary cluster; 2–4, secondary clusters

^cNumber of premises with predicted backyard bird ownership

^dRadius is zero as there is only one census block in the cluster

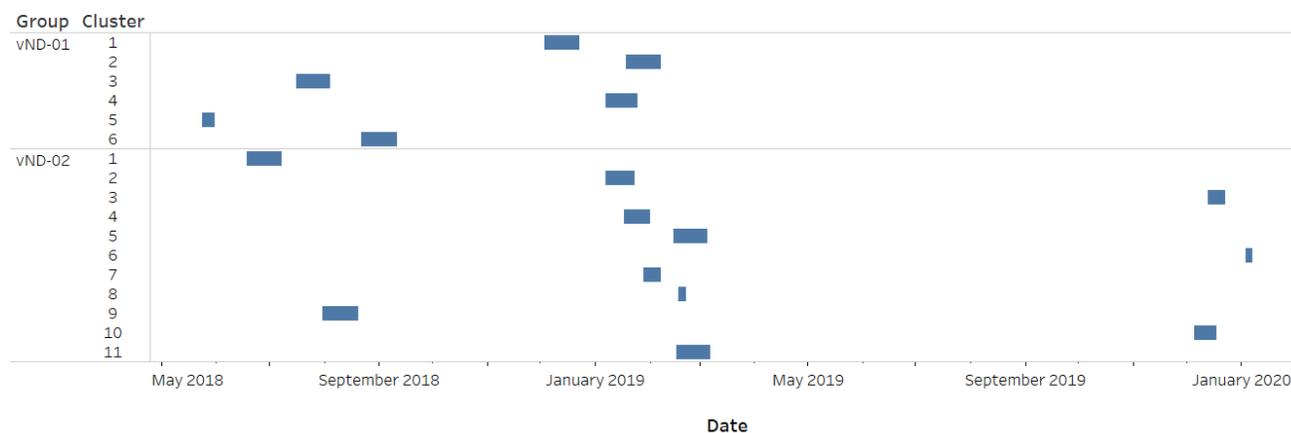


Figure 24. Time period of occurrence of spatiotemporal clusters of vND-01 and vND-02 in California from 16 May 2018 to 22 February 2020. All clusters were statistically significant at the $p < 0.05$ level. Clusters are displayed in descending order of statistical significance, with cluster 1 representing the primary (or, most likely) cluster.

Conclusions

Results identified specific geographic areas at the census block level of statistically significant spatial and spatiotemporal disease clustering. The primary spatial and spatiotemporal clusters were located within Riverside and San Bernardino counties, identifying these areas as the location of the highest occurrence of vND cases detected from 16 May 2018 to 22 February 2020. Further, these clusters occurred during the first seven months of the outbreak, corresponding to the increase in infected premises that were detected during this time as well as further disease spread over the next year within the RQA. Of the 17 statistically significant spatiotemporal clusters, 12 occurred during the months between December and March. This time period corresponds to when the fighting gamebird season occurs, as well as when resources to support the outbreak were decreased due to a federal government shutdown (December 2018 – January 2019) and following a three month time period of no newly detected cases (August – November 2019).

This approach has some limitations. vND cases may be underreported, which can result in misclassification of cases and non-cases. In addition, actual data of true backyard bird ownership in the outbreak area remains limited. We used an estimated population at risk based on the predicted number of premises of backyard ownership using 2010 census block data. As such, the true number of premises with backyard/exhibition birds used in this analysis may be under- or overestimated, resulting in the number of detected clusters and estimated risk to be over or underestimated.

In conclusion, the identification of significant spatial and spatiotemporal clustering patterns of vND in California from May 2018 to February 2020, support control strategies of targeting high risk areas for disease spread with increased response efforts in order to maximize the effectiveness of disease response strategies and control the outbreak.

G. Measuring the Spatial Dependence of Virulent Newcastle Disease Transmission Risk

Global spatial clustering methods can be used to evaluate the tendency of virulent Newcastle disease (vND) positive premises to occur closer together in spatial distance and time than would be expected by chance. Evaluating the extent of spatial clustering provides insights into the spatial scale of disease

transmission and mechanisms of disease spread, thereby informing optimal disease response strategies. When these analyses are informed by genetic and temporal data to identify likely related and unrelated infected premises, the extent of spatial clustering can be evaluated even when knowledge of the underlying population distribution is unknown (Lessler et al., 2016). The analyses presented here aimed to measure the spatial dependence of vND transmission risk over different temporal scales to inform surveillance and control strategies for the current outbreak in California.

Methods

In this analysis, data on vND infected premises from the current outbreak in California were obtained from USDA's Emergency Response Management System (EMRS). Only confirmed vND positive premises that had full genomic sequence data from USDA's National Veterinary Services Laboratories (NVSL) and were located within the Southern California vND Regional Quarantine Area, which encompasses Los Angeles County and parts of Riverside and San Bernardino Counties, were included in the analyses (CDFA, 2019). Results from the phylogenetic analysis of the genomic sequence data were used to determine the genetic relatedness between infected premises. Independent, commercial, and backyard/exhibition poultry operations were included in the analyses.

The spatial clustering statistic, τ , was used to measure the spatial dependence of vND transmission risk (Lessler et al., 2016). The τ -statistic is interpreted as a relative risk of a premises at a specified spatial distance from a vND infected premises also being infected, versus the risk of a premises located anywhere within the Regional Quarantine Area being infected. It is calculated as:

$$\hat{\tau}(d_1, d_2) = \frac{\hat{\pi}(d_1, d_2)}{\hat{\pi}(0, \infty)}$$

where $\hat{\pi}(d_1, d_2)$ estimates the probability that a vND infected premises occurs within a certain distance range (i.e., between d_1 and d_2) of another infected premises. Where spatial clustering exists, τ will be greater than 1. Geographic coordinates of vND infected premises were used to determine their distances apart. Values of $\tau(d_1, d_2)$ were calculated at 500-meter (m) wide windows centered from 250 m to 10 km in 500 m increments.

The earliest date between the reported onset of clinical signs, diagnostic sample date, and presumptive positive date was used for estimating τ at different temporal scales. The time period of main interest was infected premises that occurred within 21 days of each other, which is the maximum length of the vND incubation period¹⁵ (OIE, 2012). The relative risk of infected premises within different distance ranges was also evaluated for 42 days, or double the incubation period, and 120 days, which is the minimum fallow period for vND virus elimination for outdoor premises (USDA, 2018).

The significance of spatial clustering was assessed using bootstrapping simulation (1,000 iterations). The null distribution of the τ -statistic was obtained by randomly permuting the locations of vND infected premises and calculating the τ -statistic after each permutation. Similarly, confidence intervals for the τ -statistic were obtained using bootstrapping simulation. No comparisons were made between an individual premises and itself for the simulations (Gatrell et al., 1996, Lessler et al., 2016).

¹⁵ The incubation period is defined as the time period between when a flock becomes infected and when clinical signs appear.

Direct local and long-distance vND spread between premises were evaluated by calculating the Euclidean distance between adjacent premises and infected zones¹⁶, respectively. Parcel data of all premises within the outbreak area were obtained from the Southern California Association of Governments (SCAG, 2016). Premises immediately adjacent to an infected premises (i.e., premises sharing a fence-line/border or immediately adjacent across a residential street) were identified and the distances between the centroid and edge of the resulting adjacent premises patches were calculated. The distances between infected zones were calculated based on the minimum distance between premises within different zones. All data analyses were performed in R (v.3.4.3) and ArcGIS (v. 10.5.1).

Results

The analysis included 342 infected vND premises detected between 16 May 2018 and 1 May 2019 and located within the California Regional Quarantine Area. Based on the phylogenetic analysis (see Section II, Part A: Phylogenetic Analysis and Diagnostics), spatial clustering analyses were performed separately based on the two main vNDV sub-groups (vNDV-01 and vNDV-02) since premises within the same sub-group are genetically related, and premises between sub-groups are genetically unrelated. Sub-groups vNDV-01 and vNDV-02 contained a total of 129 and 213 premises, respectively.

The mean (standard deviation) parcel size of a vND infected premises was 100.78 (52.31) m in length (range: 22.19 – 337.27 m) and 37.18 (28.18) m in width (range: 3.05 – 204.40 m). The mean (standard deviation) parcel patch size of premises adjacent to an infected premises was 206.94 (100.0) m in length (range: 34.13 – 615.35 m) and 114.29 (62.92) m in width (range: 12.96 – 387.04 m). The mean (standard deviation) infected zone size was 5.61 (4.73) km in length (range: 1.21 – 21.60 km) and 3.32 (2.79) km in width (range: 0.83 – 13.08 km). The mean (standard deviation) minimum distance between infected zones was 6.03 (4.31) km (range: 1.87 – 20.62 km).

Strong and statistically significant spatial clustering was observed among adjacent premises, consistent with local vND spread between premises (Figure 25). The probability that a premises located within 250 m (\pm 250 m) and 21 days of another infected vND premises was 3.99 (95 percent CI: 3.21 – 4.72) and 2.78 (95 percent CI: 2.35 – 3.24) times greater for vNDV-01 (shown in blue, Figure 25) and vNDV-02 premises (shown in brown, Figure 25), respectively, than the probability that any premises within the California Regional Quarantine Area was positive for the same vNDV sub-group. For vNDV-01 premises, there is an increased probability ($\tau > 1$) of premises being infected at distances up to 9.5 km ($\tau = 1.71$, CI: 0.50 – 3.17) from another infected premises, indicating vND spread over long distances and between zones.¹⁷ However, this probability is only statistically significant at distances up to 4.5 km ($\tau = 2.08$, CI: 1.12 – 3.19), which suggests that the majority of vND spread of this sub-group was within infected zones with less spread between zones. For vNDV-02 premises, there is an increased probability of premises being infected at distances up to 10 km ($\tau = 1.43$, CI: 0.81 – 2.32); however, this probability is only

¹⁶ An infected zone (IZ) is a zone immediately surrounding the Infected Premises. The IZ will initially encompass the perimeter of all presumptive or confirmed positive premises and include as many of the Contact Premises or contiguous premises as required epidemiologically or logistically. The size of the IZ depends upon the disease agent and circumstances of the outbreak (USDA, 2018).

¹⁷ The τ -statistic is interpreted as the probability of a premises at the specified spatial distance from a vND infected premises also being infected, versus the probability of a premises located anywhere within the California Regional Quarantine Area being infected.

statistically significant at distances up to 2.5 km ($\tau = 2.08$, CI: 1.07 – 1.84) and at distances between 5.5 – 6.0 km ($\tau = 2.32$, CI: 1.38 – 3.22). These results indicate both local and long-distance vND spread for this sub-group. Long distance spread may be due to the movement of infected poultry or fomites out of infected areas. Overall, 76.5 percent of infected premises were located within 250 m of another infected premises (black dashed line, Figure 25). This percent increased to 87.8 percent at 500 m and to over 95 percent at 1.5 km.

Spatial clustering over longer time periods was also evaluated. For vNDV-01 premises, statistically significant spatial clustering was observed at distances up to 6.0 km for infected premises that occurred at 42 and 120 days apart ($\tau = 2.39$, CI: 1.58 – 2.98 and $\tau = 1.51$, CI: 1.39 – 1.62, respectively; see also Figure 26). For vNDV-02 premises, statistically significant spatial clustering was observed at distances up to 3.5 km for infected premises that occurred at 42 and 120 days apart ($\tau = 1.52$, CI: 1.17 – 1.91 and $\tau = 1.36$, CI: 1.18 – 1.52, respectively; see also Figure 26). Statistically significant clustering was also observed at distances between 5.0 and 8.0 km for these same time periods. These results indicate longer-term disease transmission, which may occur due to undetected, infected premises that allow for sustained disease spread over time or violations in the fallow period. Fallow period violations, in which poultry are repopulated on premises before 120 days have passed, have been documented for the current vND outbreak during inspections for fallow period compliance.

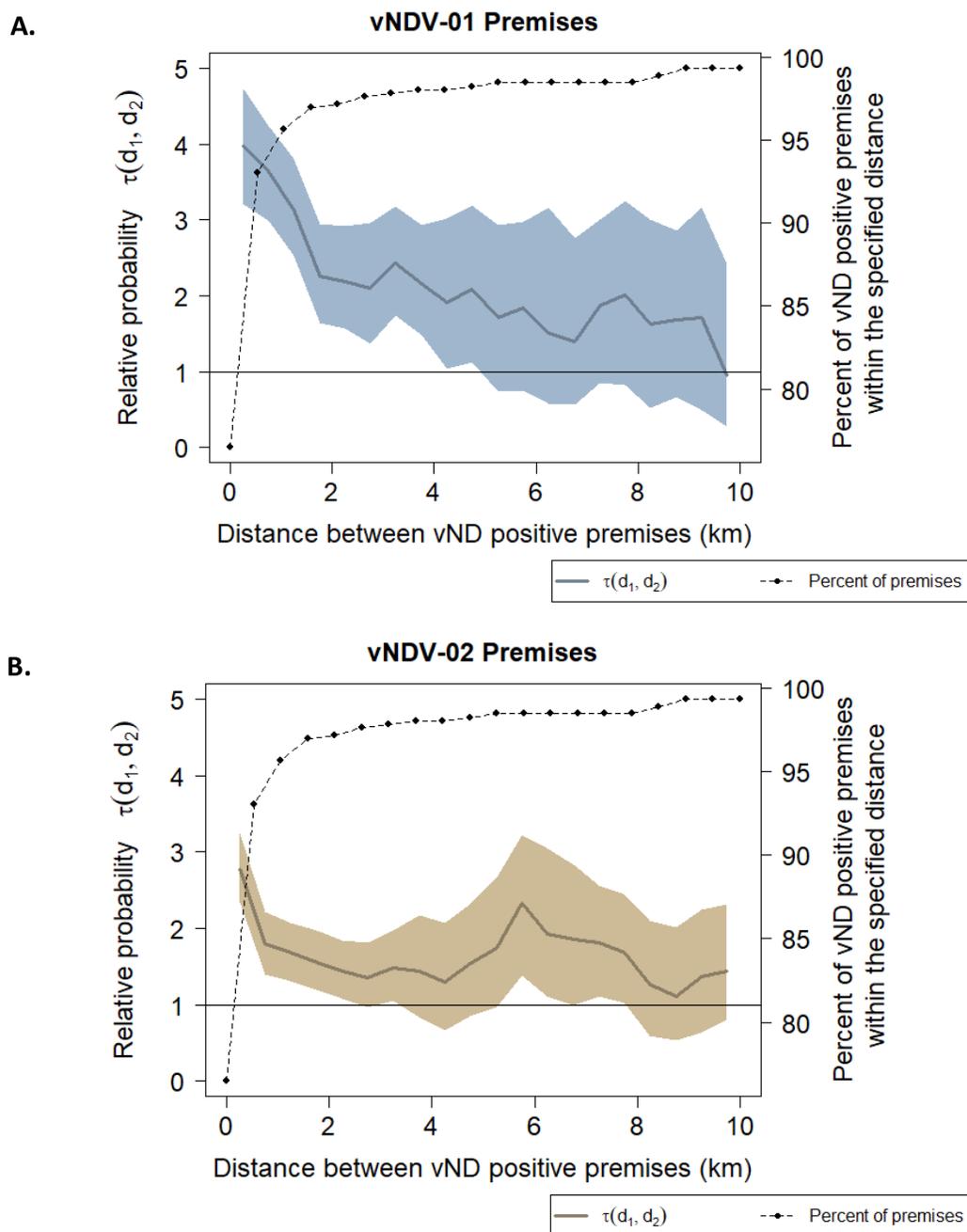


Figure 25. Spatial dependence of vND infected premises and the percent of infected premises located within the specific distance and occurring within 21 days of each other. Spatial dependence analyses were performed on premises that are genetically related based on full genomic sequencing: A) vNDV-01 sub-group infected premises, and B) vNDV-02 sub-group infected premises. The shaded area represents 95% bootstrapped confidence intervals for the spatial clustering estimates. Estimates are plotted at the mid-point of the spatial range in 500 m increments.

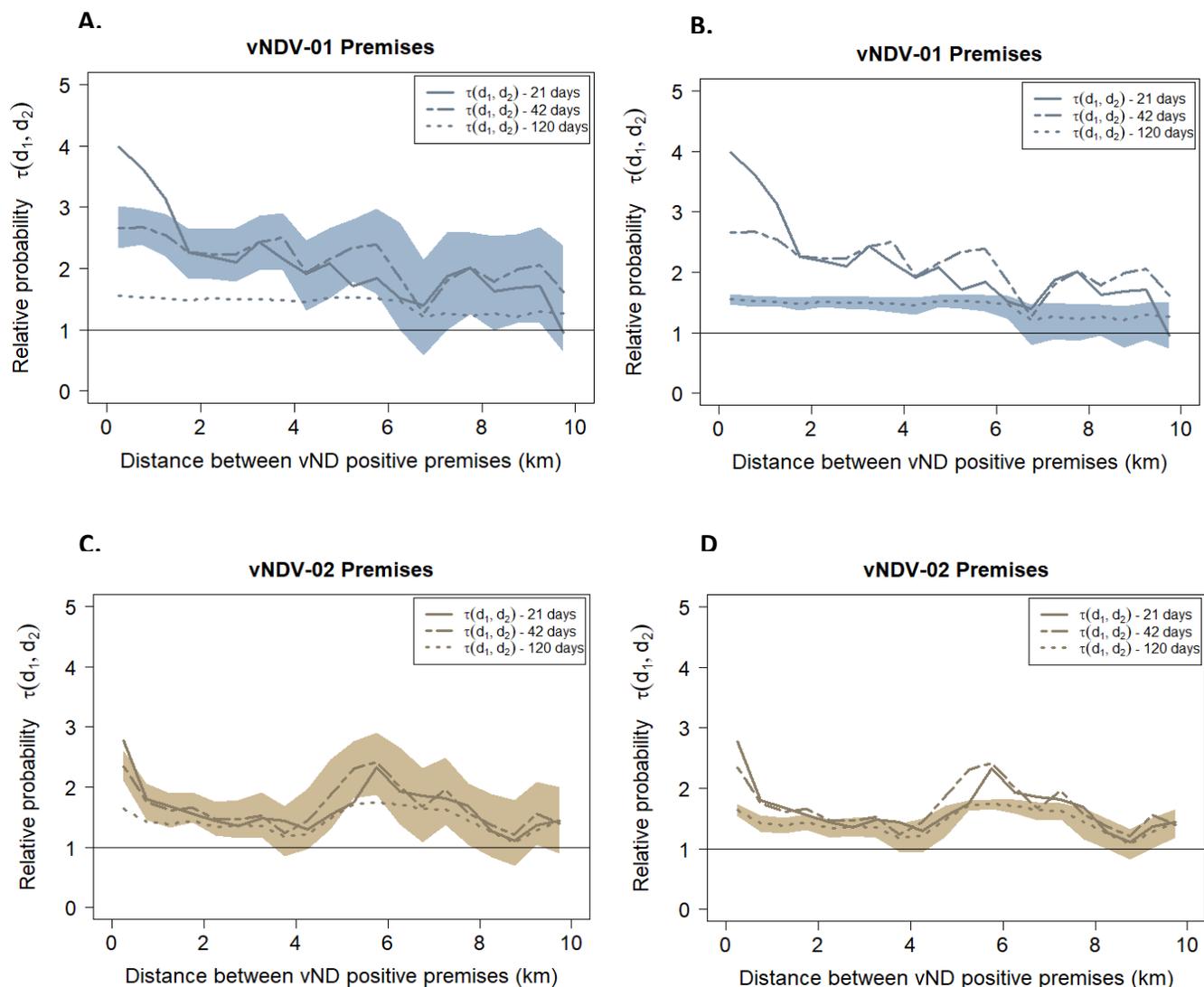


Figure 26. Spatial dependence of vND infected premises located within the specified distance and occurring within 21 and 120 days of each other. Spatial dependence analyses were performed on premises that are genetically related based on full genomic sequencing: A) and B) vNDV-01 sub-group infected premises with 95% bootstrapped confidence intervals for 42 and 120 days, respectively; C) and D) vNDV-02 sub-group infected premises with 95% bootstrapped confidence intervals for 42 and 120 days, respectively. Estimates were plotted at the mid-point of the spatial range in 500 m increments.

Conclusions

This analysis measured the spatial dependence of vND transmission risk for the current vND outbreak in California. The results indicate the highest risk of disease transmission occurs in close proximity (250 m, \pm 250 m) of infected premises for both vNDV-01 and vNDV-02 sub-groups. This risk remains statistically significantly increased up to distances between 2.5 and 4.5 km, with over 95 percent of infected premises occurring within 1.5 km of another infected premises. The results also provide additional evidence of long-distance disease spread between infected zones.

Identification of the extent of spatial clustering support surveillance and control strategies that are targeted at areas in close proximity of infected premises and at longer distances but within the same infected zone. Disease tracing information would be needed to identify areas of likely long-distance disease to other infected zones; however, genomic sequence data provide critical information on infected zones that are genetically related and therefore, can also guide disease response efforts. Given statistically significant spatial clustering was identified at distances that encompass multiple infected zones and for genetically related, infected premises occurring in different zones, this analysis supports surveillance and control strategies aimed at multiple infected zones being performed in parallel.

Implementation During Outbreak

After this analysis was completed in July 2019, the results were used to guide surveillance and depopulation efforts for backyard/exhibition premises during the vND outbreak. Mandatory depopulation of poultry was conducted within a 250 m area around infected backyard/exhibition premises. Depending on the density of backyard premises, 1 to 1.5 km surveillance areas were established around the depopulation area with mandatory surveillance of all backyard/exhibition premises conducted within a 500 m area immediately adjacent to the depopulation area. Depopulation activities prioritized backyard/exhibition premises closest to the infected premises and then worked outward (“inside-out strategy”), whereas surveillance activities prioritized backyard/exhibition premises at the outside perimeter of the mandatory surveillance area and worked inward (“outside-in strategy”). This allowed the depopulation area to be expanded if additional infected premises were found while minimizing the chance that a surveillance team would have required downtime from field activities due to testing infected, undetected premises. If additional infected premises were detected in the initial depopulation area, the area was expanded another 250 m or more, depending on the density of backyard/exhibition premises and field intelligence on sick bird observations in the area.

Between July 2019 and the end of the vND outbreak, seven depopulation areas were formed around a total of 26 infected backyard/exhibition premises. Four (57.1 percent) of the seven depopulation areas did not expand beyond the initial 250 m area and included 10 infected premises. Three (42.9 percent) of the seven depopulation areas did expand beyond the initial 250 m area; two of these were expanded by 250 m (500 m total depopulation area) and included six infected premises, whereas one was expanded by 750 m (1 km total depopulation area) and included 10 infected premises. The 1 km depopulation area was in a densely populated area of backyard/exhibition bird premises where residents had observed sick birds for several weeks.

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VI. ACKNOWLEDGEMENTS

We greatly appreciate the cooperation and support of the poultry owners for allowing us access to their properties, providing information on their biosecurity and production practices, and for their cooperation with this report.

We also appreciate the Federal and State animal health experts, as well as the many partners who assisted with these studies while serving in the incident command for this outbreak.

This report would not have been possible without a large group of researchers, epidemiologists, laboratory staff, and data scientists who participated in the various analyses, and the drafting and review of this report. We appreciate their dedication and professionalism in providing the best information possible to help bird owners reduce the risk of Newcastle disease spread.

APPENDIX A: QUESTIONNAIRE

**California Department of Food and Agriculture
CA VND 2018
Non-Commercial Premises
Virulent Newcastle Disease Epidemiology Questionnaire**

Investigator name: _____ Date of Investigation: ____/____/____

Investigator name: _____

Quarantine # _____ Date Quarantine Issued: ____/____/____

1. Name of **Premises Owner**:

(First) (MI) (Last)

2. Premises Address (location of birds):

Latitude: _____ Longitude: _____

3. Premises Owner Telephone #:

- a. Mobile: _____
b. Home: _____
c. Other: _____

If Premises Owner is the Bird Owner skip to Question 7

4. Name of **Bird Owner**:

(First) (MI) (Last)

5. Bird Owner Address: _____

6. Bird Owner Telephone #: _____

7. Other than the interviewee, how many **other** owners with birds
are on this premises:

8. How many birds do you have on the premises today? # _____

9. What percent of the adult **chickens** are: a) Roosters % _____
 b) Hens % _____

10. Which of the following birds are on the premises? Complete table below.

Type of Bird	# Adults	# Young birds	Total
Backyard Poultry	a	b	c
Exhibition Birds/gamefowl	d	e	f
Ducks/Geese	g	h	i
Other Specify j	k	l	m
Other Specify n	o	p	q

11. Which of the following animals are on the premises (potential fomites)?

- a) Livestock (Horses, Cattle, Swine, Sheep, Goats) ₁ Yes ₃ No
- b) Dogs/Cats ₁ Yes ₃ No
- c) Other (specify _____) ₁ Yes ₃ No

12. Which of the following housing types are used to house birds?

- a) Inside the home ₁ Yes ₃ No
- b) Outdoor **open** top poultry pen or enclosure ₁ Yes ₃ No
- c) Outdoor cages or coops - **fully enclosed** ₁ Yes ₃ No
- d) Individually tethered ₁ Yes ₃ No
- e) Free range ₁ Yes ₃ No
- f) Other (Specify _____) ₁ Yes ₃ No

13. Has there been an increase in illness in your birds

on your premises? ₁ Yes ₃ No

a) If yes, how many days ago did the birds first show signs of illness: _____ days

Which of the following clinical signs of illness have you observed?

Check all that apply.

- b) Not eating ₁ Yes ₃ No
- c) Coughing/gasping ₁ Yes ₃ No
- d) Depressed ₁ Yes ₃ No
- e) Twisting of the neck ₁ Yes ₃ No
- f) Paralysis ₁ Yes ₃ No
- g) Diarrhea ₁ Yes ₃ No
- h) Swellings around the eyes and neck ₁ Yes ₃ No
- i) Sudden death ₁ Yes ₃ No
- j) Other (specify _____) ₁ Yes ₃ No

14. Have there been any deaths in your birds on this premises

during the past 30 days? ₁ Yes ₃ No

a) If yes, when did the first bird die? ____/____/____

b) If yes, how many birds died in the first 7 days? # _____

c) If yes, how many birds have died in the past 7 days? # _____

15. Do you keep any birds at another premises? ₁ Yes ₃ No

a) If yes, where are the birds housed?

16. Have you brought new birds onto this premises during the past 30 days? ₁ Yes ₃ No

If Yes, list date and name the source and location of the new birds:

<u>Date</u>	<u>Source/Location</u>
____/____/____ a	_____ b
____/____/____ c	_____ d
____/____/____ e	_____ f

17. Have any of the following had contact with your birds, feed or water sources on your property in the last 30 days?

- a) Wild birds (e.g., pigeons, doves, sparrows) ₁ Yes ₃ No
- b) Neighborhood/community chickens ₁ Yes ₃ No
- c) Wild animals ₁ Yes ₃ No

18. Have any of your **birds** left these premises during the last 30 days? ₁ Yes ₃ No

If Yes, for what purposes listed below were the birds moved?

Purpose	Date	Destination (City/State)	# of birds
Sale	a	b	c
Show	d	e	f
Competition	g	h	i
Veterinary care	j	k	l
Gift/trade m	n	o	p
Other Specify q	r	s	t

If Yes, did any birds leave and then return to these premises? ₁ Yes ₃ No

19. Do you give away or sell **eggs** from this premises? ₁ Yes ₃ No

20. Do your neighbors have birds? ₁ Yes ₃ No
If No, skip to Question 23.

If Yes, please note location(s) on the map at the end of the questionnaire.

21. When not cooped, do your birds ever visit the neighbor's property? ₁ Yes ₃ No

22. Do your neighbor's birds ever come onto your property? ₁ Yes ₃ No

a) If Yes, do the neighbors birds have contact with your birds? ₁ Yes ₃ No

23. Do you have **family members or close friends** who own/keep birds? ₁ Yes ₃ No

If Yes, do any of the following situations occur (evaluating direction of exposure):

- a) Your family or friends handle birds when they visit. ₁ Yes ₃ No
- b) When visiting family/friends do you handle their birds. ₁ Yes ₃ No

24. What is the name and location of the store(s) where you get feed and supplies for your birds?

<u>Name</u>	<u>Location (City)</u>
_____ a	_____ b
_____ c	_____ d
_____ e	_____ f

25. Have the birds on your premises **today** been vaccinated with Newcastle vaccine? ₁ Yes ₂ Unsure ₃ No

Vaccine does not protect against disease!

a) If Yes, at what age(s) were your birds vaccinated with Newcastle vaccine?

26. Have you seen any dead wild birds on your premises in the last 30 days? ₁ Yes ₃ No

If Yes, what type of wild bird(s)?

_____ a	_____ b
_____ c	_____ d

Additional comments, observations and leads:

Insert Google Maps Image of the premises or draw a map and specify bird locations. Please indicate which neighbors, if any, have birds.

I _____ certify that I have _____ birds on / / @ _____
 (owner signature) (number) (date and time)

APPENDIX B: MODELING SCENARIO DESIGN

Control Activities Associated with Respective Response Options

Scenario Design Overview

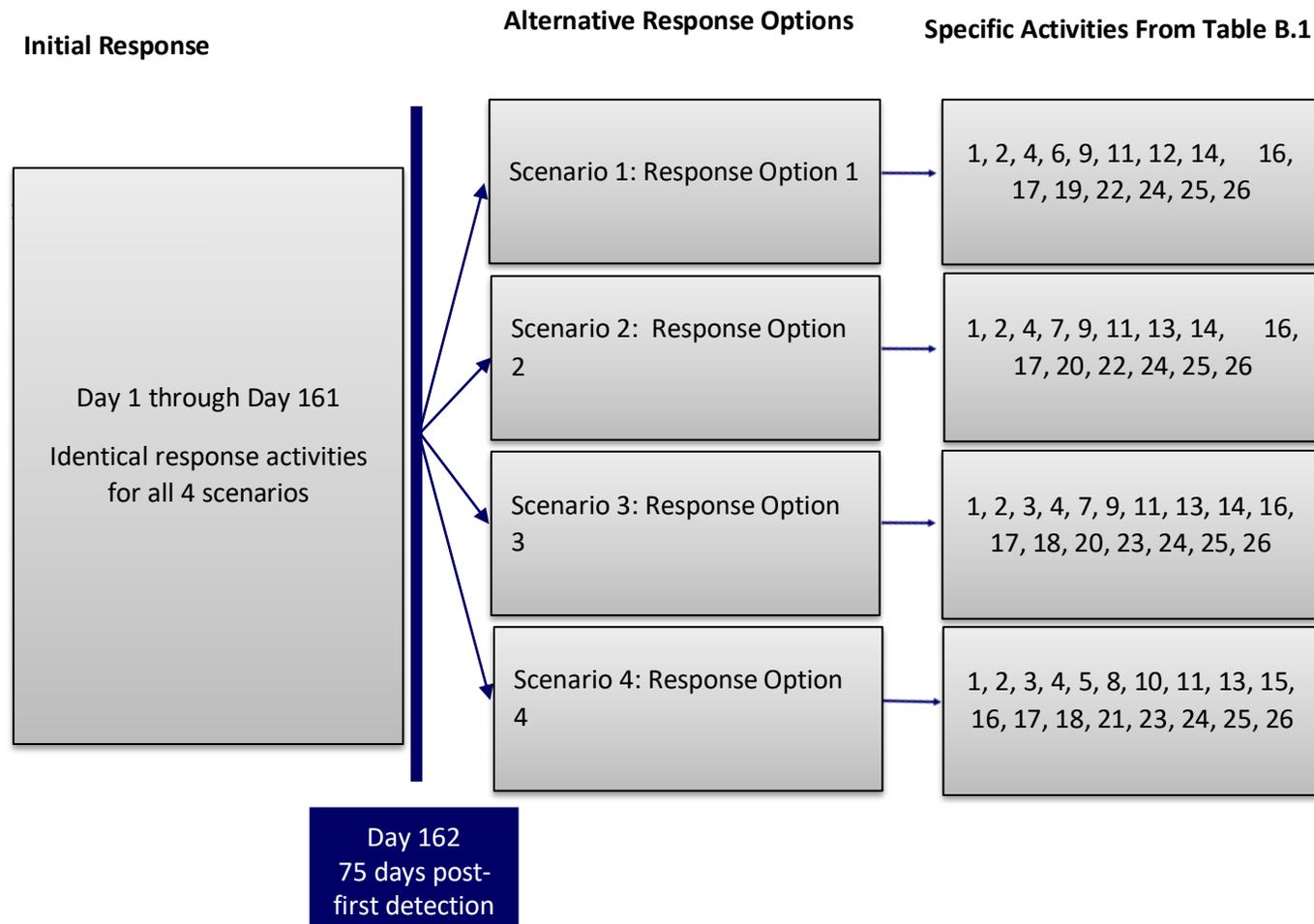


Table B1. Individual Control Activities Included in Response Options

	Control Activity	Response Options			
		1	2	3	4
1	Depopulation: detected backyard premises	x	x	x	x
2	Depopulation: backyard premises in proximity to detected backyard premises in Muscoy Zone	x	x	x	x
3	Depopulation: backyard premises in proximity to detected backyard premises in other high-risk zones (e.g., Bloomington, Fontana, Riverside)			x	x
4	Depopulation: detected commercial premises	x	x	x	x
5	Depopulation: backyard premises in proximity to all detected backyard premises				x
6	Depopulation: capacity (low – maximum of 6 backyard premises per day)	x			
7	Depopulation: capacity (medium – maximum of 10 backyard premises per day)		x	x	
8	Depopulation: capacity (high – maximum of 30 backyard premises per day)				x
9	Movement restrictions for live animal movements originating from zoned backyard premises (low capacity – 30% of high capacity)	x	x	x	
10	Movement restrictions enhanced for live animal movements originating from zoned backyard premises (high capacity)				x
11	Movement restrictions for live animal movements originating from zoned commercial premises	x	x	x	x
12	Movement restrictions for live animal movements originating from traced premises (low capacity – 50% of high capacity)	x			
13	Movement restrictions for live animal movements originating from traced premises (high capacity)		x	x	x
14	Surveillance – Passive: Sick calls – initiates active surveillance visit (low public disease awareness)	x	x	x	
15	Surveillance – Passive: Sick calls – initiates active surveillance visit (high public disease awareness – results in greater number of calls and surveillance visits)				x
16	Surveillance – Active: 1-km radial zone around detected backyard premises	x	x	x	x
17	Surveillance – Active: Irregular zone surge (Muscoy) [enhanced surveillance for backyard premises]	x	x	x	x
18	Surveillance – Active: Irregular zone surge (e.g., Bloomington, Fontana, Riverside) [enhanced surveillance for backyard premises]			x	x
19	Surveillance – Active (post-irregular zone surge): Low capacity – fewest number of backyard premises eligible for surveillance (approx. 30% of high capacity)	x			
20	Surveillance – Active (post-irregular zone surge): Medium capacity (approx. 67% of high capacity)		x	x	
21	Surveillance – Active (post-irregular zone surge): High capacity – greatest number of backyard premises eligible for surveillance				x
22	Surveillance – Active: baseline response time after zone formation (approx. 2X longer response time than for enhanced response)	x	x		
23	Surveillance – Active: enhanced response time after zone formation			x	x
24	Surveillance – Active: weekly to bi-weekly sampling of commercial premises	x	x	x	x
25	Tracing live animal movements originating from detected farms (movements occurred prior to detection)	x	x	x	x
26	Tracing indirect contacts originating from detected commercial farms (movements occurred prior to detection)	x	x	x	x

APPENDIX C. PREDICTED DISEASE MORTALITY AND INFECTION PREVALENCE UNDER ADDITIONAL FLOCK SIZE AND VACCINATION SCENARIOS

In this appendix, results are provided for unvaccinated and vaccinated backyard flocks housing 50 birds, unvaccinated and vaccinated commercial flocks housing 5000 birds, and unvaccinated commercial flocks housing 20,000 birds. The trends in the results for these additional scenarios are consistent with those noted in the main text, including slower spread and lower mortality in vaccinated flocks, and longer amounts of time required to reach specific cumulative mortality levels in larger flocks.

Input parameters to the transmission model were estimated from inoculation study data and data from the current outbreak. A summary of the parameters is given in Table C1. Model parameters for the latent and infectious period distributions in unvaccinated flock scenarios were estimated using available experimental inoculation studies for the CA/2018 and other vND strains (Dimitrov et al., 2019) as described in Section V: Part C. The infectious period parameters for vaccinated flocks were estimated from experimental data presented in Miller et al. (2013) from vaccinated SPF chickens and contact birds. The latent period distribution from the unvaccinated flock scenario was also used in the vaccinated flock scenario due to a lack of adequate data on vaccinated birds.

The contact rate in the vaccinated commercial flock scenario and the mortality proportion in vaccinated flocks were based on estimates from the time of introduction analysis in Section V: Part E. The contact rate in the unvaccinated commercial flock scenario was estimated as described in Section V: Part C. Due to greater uncertainty, the contact rate distributions were widened in the backyard flock scenarios. All birds infected in the unvaccinated bird scenarios were assumed to die from the disease.

Table C1. Transmission model input parameters used in the estimation of infection prevalence and disease mortality over time in vND infected vaccinated and unvaccinated flocks

Parameter Name	Description	Distribution
Adequate Contact Rate	Daily average number of contacts a bird has with other birds that are sufficient to transmit infection	Vaccinated commercial flocks: PERT (min = 0.90, mode = 1.20, max = 2.50)
		Unvaccinated commercial flocks: Uniform (min = 1.7, max = 4.0)
		Vaccinated backyard flocks: PERT (min = 0.5, mode = 1.20, max = 2.50)
		Unvaccinated backyard flocks: Uniform (min = 1.00, max = 4.00)
Latent Period Length Distribution	Length of the latent period	Vaccinated and unvaccinated flocks: Gamma (shape = 1.00, scale = 0.39); mean = 0.39 days; variance = 0.15 days ²
Infectious Period Length Distribution	Length of the infectious period	Vaccinated flocks: Gamma (shape = 2.30, scale = 2.48); mean = 5.68 days; variance = 14.07 days ²
		Unvaccinated flocks: Gamma (shape = 13.07, scale = 0.33) mean = 4.34 days; variance = 1.44 days ²
Mortality Proportion	Proportion of birds that die in a barn following exposure to vND	Vaccinated flocks: Uniform (min = 0.030, max = 0.047)
		Unvaccinated flocks: 100% mortality
Proportion Immune	Proportion of birds in a barn that are immune to vND following vaccination	Uniform (min = 0.00, max = 0.04)

Additional model scenarios for backyard flocks

Predicted mortality and disease prevalence in a 50-bird unvaccinated backyard flock

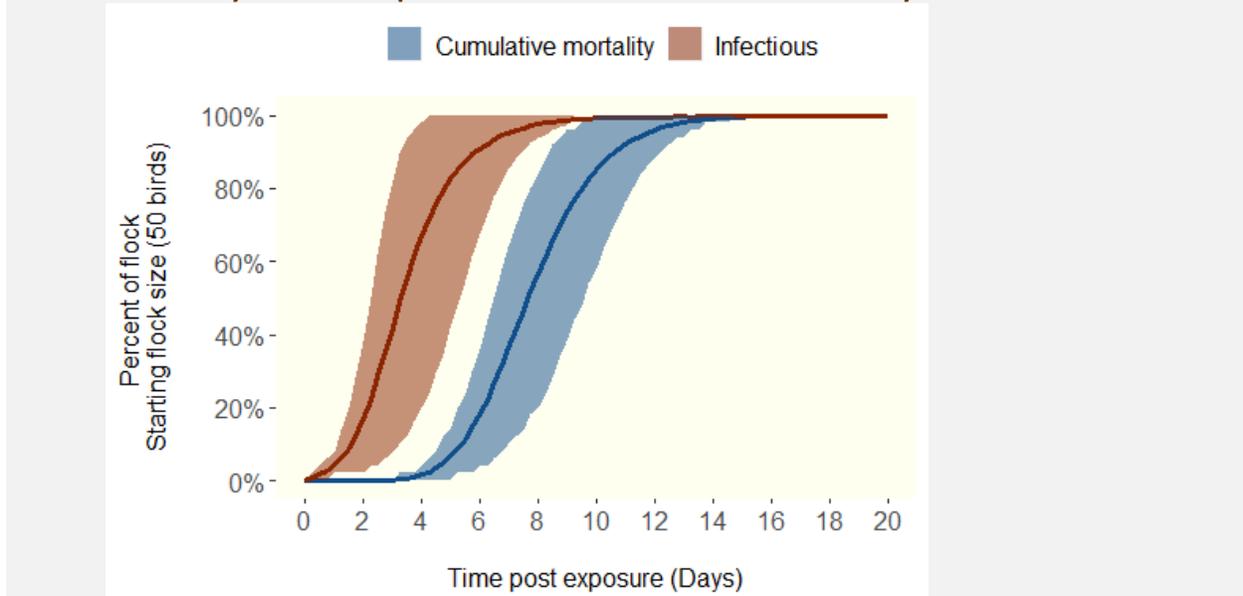


Figure C1. Predicted cumulative mortality percent and prevalence of infectious birds on various days post exposure in a 50-bird unvaccinated backyard flock using baseline vND spread parameters. Shaded area represents the 90% prediction interval for each variable.

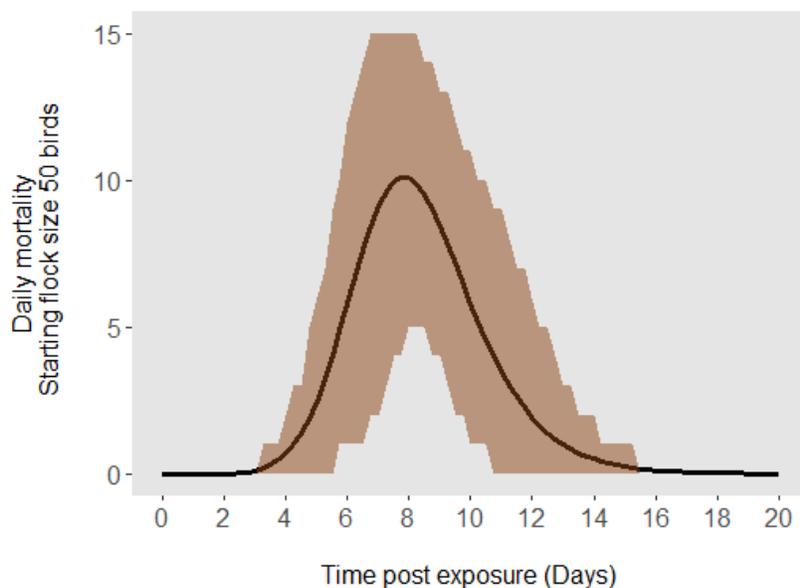


Figure C2. Predicted daily mortality on various days post exposure in a 50-bird unvaccinated backyard flock using baseline vND spread parameters. Shaded area represents the 90% prediction interval.

Table C2. Predicted days post onset of infectiousness to reach various cumulative mortality levels in a 50-bird unvaccinated backyard flock using baseline vND spread parameters

Cumulative Percent Mortality	Predicted number of days post onset of infectiousness to reach this cumulative mortality percent. median (90% Prediction Interval)
10	5.3 (4-7)
20	6 (4.8-8)
30	6.6 (5.2-8.8)
40	7 (5.5-9.2)
50	7.4 (6-9.8)
60	7.8 (6.2-10.2)
70	8.4 (6.8-11)
80	8.8 (7.2-11.5)
90	9.5 (7.8-12.5)

Predicted mortality and disease prevalence in a 50-bird vaccinated backyard flock

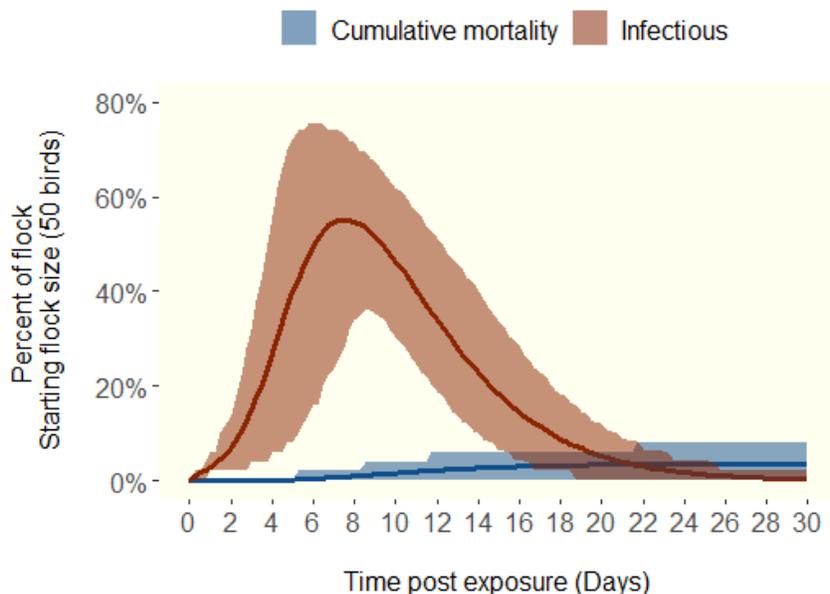


Figure C3. Predicted cumulative mortality percent and prevalence of infectious birds on various days post vND exposure in a 50-bird vaccinated backyard flock. Shaded area represents the 90% prediction interval for each variable.

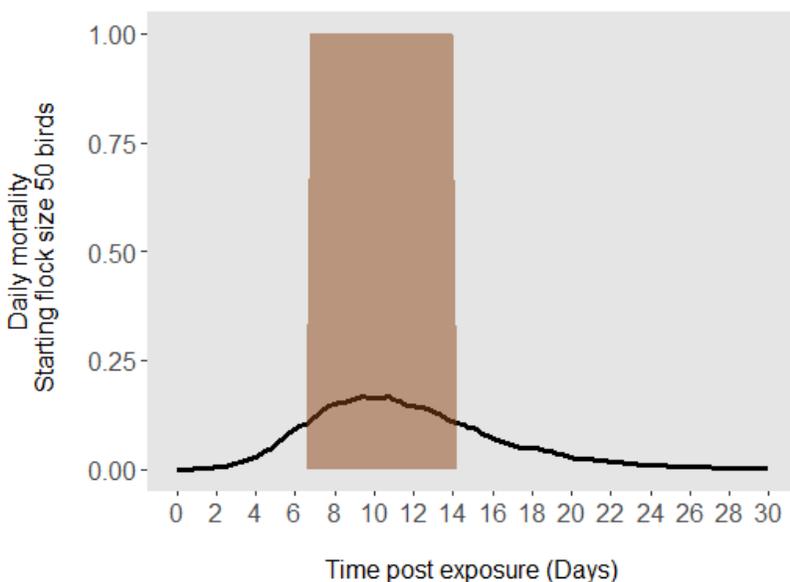


Figure C4. Predicted daily mortality on various days post vND exposure in a 50-bird vaccinated backyard flock. Shaded area represents the 90% prediction interval.

Table C3. Predicted days post onset of infectiousness to reach various cumulative mortality levels in a 50-bird vaccinated backyard flock using baseline vND spread parameters

Cumulative Percent Mortality	Percent of simulation iterations in which this cumulative mortality is reached	Predicted number of days post onset of infectiousness to reach this cumulative mortality percent. median (90% Prediction Interval)
2	80.32	8.8 (3.5-16.5)
4	53.13	11.6 (6-20)
6	27.63	13.2 (7.5-21.5)
8	10.8	14.1 (8.2-22.2)

Additional model scenarios for commercial flocks

Predicted mortality and disease prevalence in a 5000-bird unvaccinated commercial flock

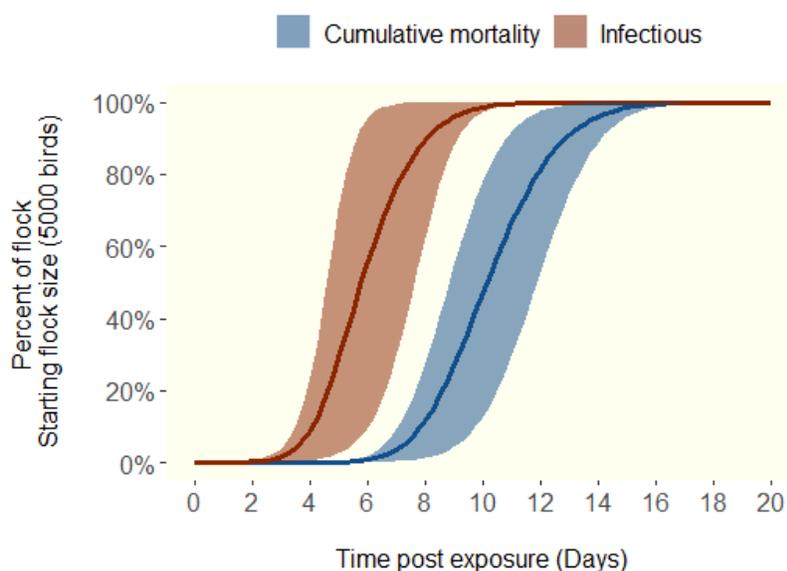


Figure C5. Predicted cumulative mortality percent and prevalence of infectious birds on various days post vND exposure in a 5000-bird unvaccinated commercial flock. Shaded area represents the 90% prediction interval for each variable.

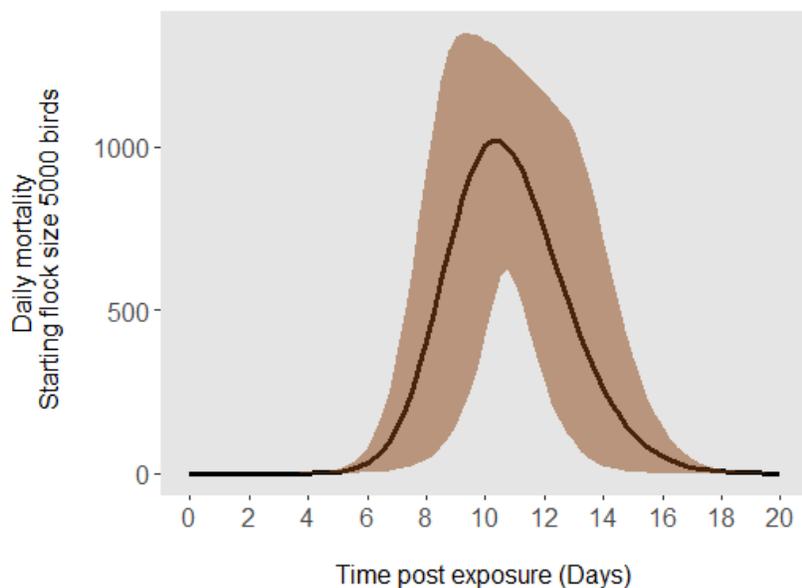


Figure C6. Predicted daily mortality on various days post vND exposure in a 5000-bird unvaccinated commercial flock. Shaded area represents the 90% prediction interval.

Table C4. Predicted days post onset of infectiousness to reach various cumulative mortality levels in a 5000-bird unvaccinated commercial flock using baseline vND spread parameters

Cumulative Percent Mortality	Predicted number of days post onset of infectiousness to reach this cumulative mortality percent. median (90% Prediction Interval)
10	7.9 (6.5-9.8)
20	8.6 (7.2-10.5)
30	9 (7.5-11)
40	9.4 (8-11.5)
50	9.8 (8.2-12)
60	10.2 (8.8-12.2)
70	10.6 (9.2-12.8)
80	11.2 (9.5-13.5)
90	11.9 (10.2-14.2)

Predicted mortality and disease prevalence in a 20,000-bird unvaccinated commercial flock

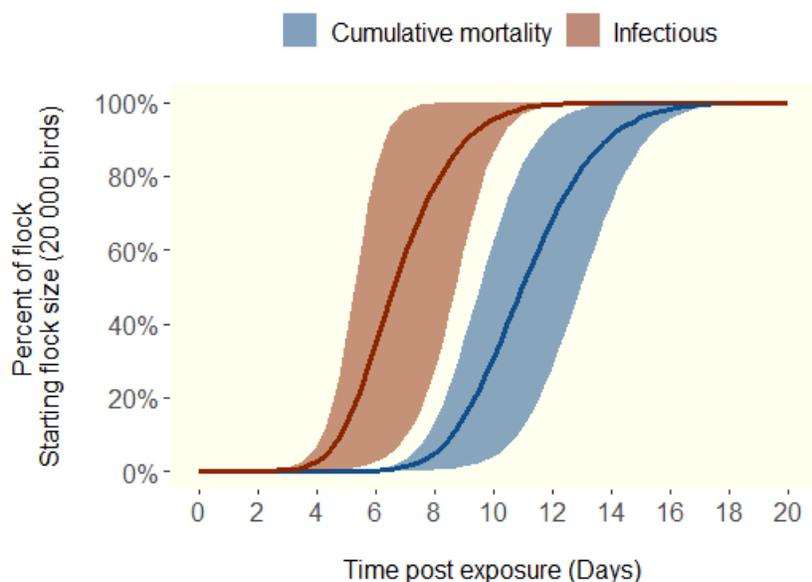


Figure C7. Predicted cumulative mortality percent and prevalence of infectious birds on various days post vND exposure in a 20,000-bird unvaccinated commercial flock. Shaded area represents the 90% prediction interval for each variable.

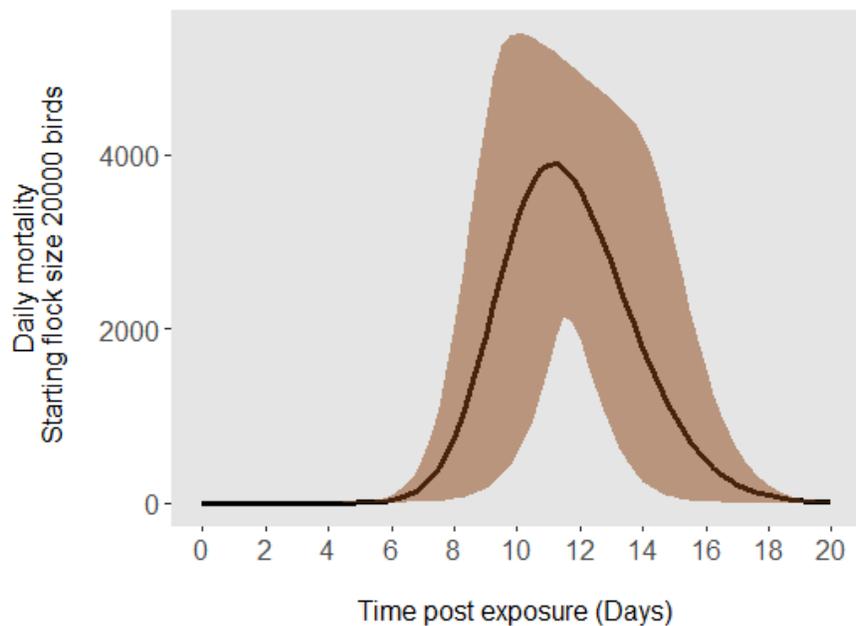


Figure C8. Predicted daily mortality on various days post vND exposure in a 20,000-bird unvaccinated commercial flock. Shaded area represents the 90% prediction interval.

Table C5. Predicted days post onset of infectiousness to reach various cumulative mortality levels in a 20,000-bird unvaccinated commercial flock using baseline vND spread parameters

Cumulative Percent Mortality	Predicted number of days post onset of infectiousness to reach this cumulative mortality percent. median (90% Prediction Interval)
10	8.8 (7.2-10.8)
20	9.4 (8-11.5)
30	9.9 (8.2-12.2)
40	10.3 (8.8-12.5)
50	10.7 (9-13)
60	11.1 (9.5-13.5)
70	11.5 (9.8-14)
80	12 (10.2-14.5)
90	12.8 (11-15.2)

Predicted mortality and disease prevalence in a 5000-bird vaccinated commercial flock

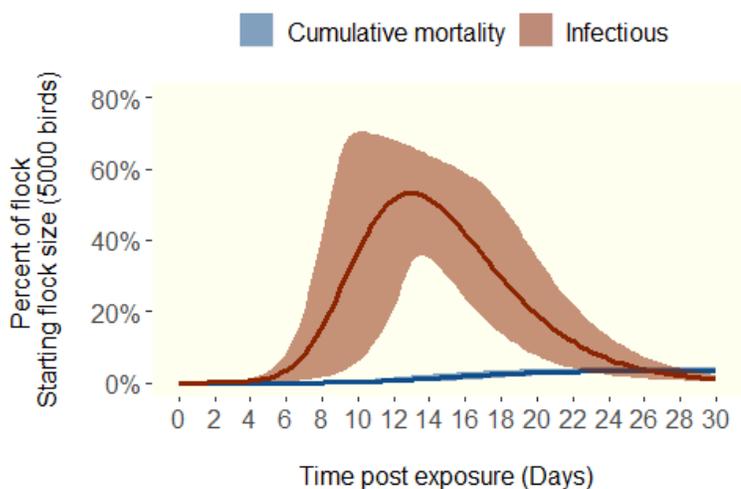


Figure C9. Predicted cumulative mortality percent and prevalence of infectious birds on various days post exposure in a 5000-bird vaccinated commercial cage free layer flock using baseline vND spread parameters. Shaded area represents the 90% prediction interval for each variable.

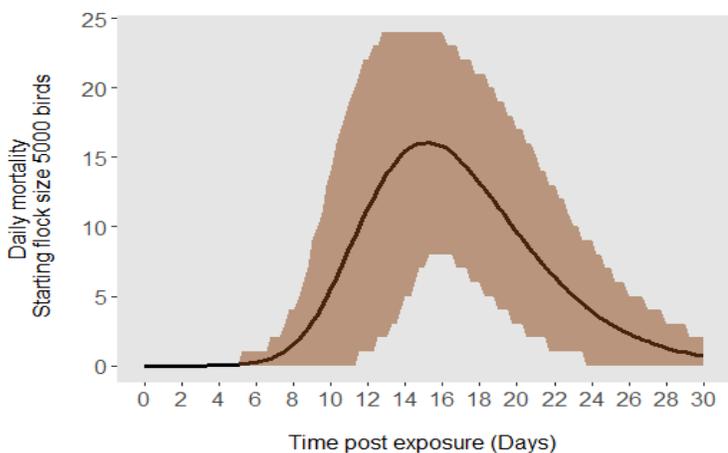


Figure C10. Predicted daily mortality on various days post exposure in a 5000-bird vaccinated commercial cage free layer flock using baseline vND spread parameters. Shaded area represents the 90% prediction interval.

Table C6. Predicted days post onset of infectiousness to attain various cumulative mortality levels in a 5000-bird vaccinated commercial cage free layer flock using baseline vND spread parameters

Cumulative Percent Mortality	Percent of simulation iterations in which this cumulative mortality is reached	Predicted number of days post onset of infectiousness to reach this cumulative mortality percent. median (90% Prediction Interval)
2	95.69	15.9 (12.2-20)
2.5	95.17	17.6 (13.5-22.8)
3	83.86	19.6 (15-25.8)
3.5	56.66	21.1 (16.2-27.2)
4	27.45	22.8 (18-28.2)
4.5	5.67	24.3 (19.2-28.8)
5	0	NA

APPENDIX D. TECHNICAL DETAILS FOR ESTIMATING THE TIME OF VND INTRODUCTION IN COMMERCIAL VACCINATED LAYER FLOCKS

A. Overview

We used an approximate Bayesian computation (ABC) approach together with Monte Carlo simulation to estimate the time of vND introduction. Specifically, we used the ABC-MCMC function of the R package EasyABC in our estimation. This function implements the methods proposed by Marjoram et al., 2003 where the parameter space is explored via a modified Metropolis-Hastings algorithm that does not involve likelihood calculation (Marjoram, 2003). The function also implements improvements proposed by Wegmann et al., (2009) to perform an automatic calibration step to determine a tolerance threshold for the goodness of fit metric representing the deviation between model predictions and the observed data.

The algorithm begins with the calibration step where the goodness of fit measure is calculated for a specified number of model iterations simulated with the prior distributions. The threshold value for the goodness of fit metric ψ_{max} is then set according to the input tolerance quantile (default 0.01). In the next step, the new values for parameters are generated according to a uniform proposal distribution and the iteration is accepted if $\psi < \psi_{max}$. The model is then run until the required number of samples is collected. The approximate posterior distribution for the parameters is calculated from the parameter values in the selected iterations.

B. Estimating prior distributions

The prior distributions for the model parameters were based on estimates from experimental inoculation studies as well as the current outbreak data. A summary of the prior distributions used in the transmission model is given in Table D1. The distributions for the lengths of the infectious and latent periods in individual birds were estimated from data in Miller et al. 2013 consisting of vaccinated SPF chickens and contact birds (Miller, 2013). Parameters estimated for these distributions, assumed to be gamma distributed, were estimated using a maximum likelihood approach. Analysis of transmission data from Miller et al. 2013 indicated a contact rate in the range of 0.53 to 2.4 contacts per day for vaccinated flocks. Given the greater uncertainty regarding the transmission rate in commercial vaccinated poultry flocks, we used a uniform (0.2, 5) contacts per day as the prior distribution.

The mortality among vaccinated birds in Miller et al. 2013 varied widely depending on the time between vaccination and challenge (from 40 percent to 0 percent mortality for birds challenged at days 3, 10 and 21 post vaccination). We used a uniform (0, 0.12) prior for the probability that a vaccinated and infected bird dies from disease. The transmission model allows for a proportion of the birds to be completely immune due to vaccination. There is a considerable uncertainty regarding this model parameter in field flocks. Given that most of the infected barns in the premises had older aged birds, we used a uniform (0, 0.04) distribution for the proportion of birds that are immune.

The input parameters for the mean and standard deviation of the normal mortality and egg production were estimated directly from the available production data for each barn. There is substantial uncertainty in the egg production parameters as only 12 days of data were available for each barn, while several weeks of mortality data were available.

We used wide ranges for the egg production rates in healthy and vND infected hens to account for the uncertainty in these parameters. For example, for barn A, the egg production was fairly constant at 70 percent for a few days before dropping off. We used a prior of uniform (0.68, 0.72) for the egg production in healthy hens for barn A. The ending egg production on the last day of production data for barn A was 28 percent. We used a uniform (0.2, 0.3) distribution as the prior for the egg production rate in vND infected hens. The egg production drops in barns B and C were much milder compared to barn A. Egg production fell in these barns from about 70 percent to 50-60 percent. A uniform (0.64, 0.74) prior was used for egg production in healthy hens in barns B and C, while a uniform (0.4, 0.55) distribution was used for egg production in sick hens. There was almost no drop in egg production for barn D with the egg production around 90 percent on all days. A uniform (0.90, 0.94) distribution was used for egg production in healthy hens and a uniform (0.80, 0.90) distribution was used for egg production in sick hens in this barn.

More than 3 months of normal mortality data were available for barns A, B, and C. We estimated the normal mortality for these barns using a linear model for 30 days prior to 11/5/2018. The linear model is useful to account for the increasing trend in daily mortality with age under routine production. The normal mortality for barn D was estimated from other barns with similar ages of birds which tested negative on 1/3/2019 and had no pattern of drop in egg production or increased mortality. The input distributions related to mortality and egg production for the four barns are summarized in Table D2.

C. Goodness of Fit measure calculation

The goodness of fit measure metric ψ consisted of the sum of the mortality cost, the egg production cost and the diagnostic testing cost. The mortality cost D_m was calculated as the average sum of squared normalized residuals between the model's predicted mortality and the data as shown in appendix Equation 1. Here M_{obs} and M_{sim} are the observed and simulated mortalities, σ_m is the standard deviation of normal mortality and N is the number of days of mortality data. Note that the residual sum of squares was also used for summary statistic calculation in other studies for parameter estimation from outbreak data (Guinat, 2018). Similarly, the egg production cost D_e was defined as the average sum of squared normalized differences between the model's predicted egg production and the data.

$$D_m = \frac{1}{N} \sum_{t_i}^{t_m} \left(\frac{M_{obs}(t) - M_{sim}(t)}{\sigma_m} \right)^2 \quad \text{Appendix Equation 1}$$

Table D1. Input prior distribution parameters used in the ABC approach to estimate the contact rate and time of virus introduction.

Parameter Name	Description	Distribution
Adequate Contact Rate	Daily average number of contacts a bird has with other birds that are sufficient to transmit infection	Uniform (min = 0.2, max = 5.0)

Latent Period Length Distribution	Length of the latent period	Gamma (shape = 1.00, scale = 0.39); mean = 0.39 days; variance = 0.15 days ²
Infectious Period Length Distribution	Length of the infectious period	Gamma (shape = 2.30, scale = 2.48); mean = 5.68 days; variance = 14.07 days ²
Mortality Proportion	Proportion of birds that die in a barn following exposure to vND	Uniform (min = 0, max = 0.11)
Proportion Immune	Proportion of birds in a barn that are immune to vND following vaccination	Uniform (min = 0.00, max = 0.04)

Table D2. Input related to mortality and egg production in the ABC approach to estimate the contact rate and time of virus introduction.

Input parameter	Parameter values used for different barns			
	Barn A	Barn B	Barn C	Barn D
Egg production in healthy hens	0.68 - 0.72	0.64 - 0.74	0.64 - 0.74	0.90 - 0.94
Egg production in vND infected hens	0.2 - 0.3	0.4 - 0.55	0.4 - 0.55	0.8 - 0.9
Normal mortality fraction	0.000638	0.000490	0.000463	0.000606
Standard deviation of normal mortality fraction	0.000373	0.000236	0.000214	0.000554

The diagnostic testing cost was set to 0 if all the test results in an iteration matched the observed test results or to a large value (15) otherwise. Given this cost structure, only the iterations where the simulated and observed test results matched were selected in the Markov chain in the simulation results.

D. Model Implementation and coding validation

The disease transmission model was coded in the languages R and C. The R package EasyABC was used to estimate the posterior distribution. The number of iterations run for the distance threshold calibration was set to 20000. The model was run for 3000-6000 iterations with 1/50 thinning to account for the higher autocorrelation. For validation of the ABC approach, a forward simulation method was developed to estimate the time of virus introduction and adequate contact rate for barn 48. The forward simulation method consisted of comparing data simulated from the stochastic disease transmission model to the egg production, mortality, and diagnostic testing data from barn 48 for candidate virus introduction date and contact rate pairs evaluated across a grid. For each transmission model iteration an indicator variable for whether the simulated data fell within a certain distance of the egg production and mortality data was multiplied by the likelihood of observing the diagnostic test results given the simulated data. These values were averaged across 10 000 iterations performed for each contact rate and time of virus introduction pair to estimate a posterior likelihood. Table D3 compares the median and 95 percent C.I. time of virus introduction estimated from the forward simulation method with the estimates from the ABC method. The results suggest the two methods are in reasonable agreement, which is evidence that the ABC method was implemented accurately and run for a sufficient number of iterations to achieve convergence.

Table D3. The median and 95% C.I. for the time of virus introduction estimated from barn 48 egg production, mortality, and diagnostic testing data from two estimation approaches.

Estimation method	Time of introduction
	Estimated median (95% C.I.)
ABC	12/23/2018 (12/17/2018 -12/26/2018)
Forward simulation	12/20/2018 (12/15/2018 – 12/23/2018)

Results

The results for the estimated day of vND introduction and the adequate contact rate for the four barns are shown in Table D4. The model fits to the observed egg production rate and daily mortality across different barns are shown in Figures D1-D8. The interval for the estimated time of introduction is the narrowest for barn A which had the highest drop in egg production and the most elevated mortality above baseline. There is a greater uncertainty in the times of introduction and the contact rate for other barns given the mild drops in egg production and mild elevation in mortality. These barns also have a lower adequate contact rate (which determines the rate of within barn disease spread) which leads to a greater uncertainty in the estimated time of introduction.

Table D4. Estimated time of introduction and adequate contact rate.

Barn	Time of introduction	Adequate contact rate
	Estimated mode, median, (95% C.I.), (90% C.I)	Estimated mode, median, (95% C.I.), (90% C.I)
A (cage free layers)	12/25/2018, 12/23/2018	1.42, 1.86 (1.02-4.72)
	(12/17/2018 -12/26/2018)	(1.09-4.56)
	(12/18/2018 -12/26/2018)	
B (cage free layers)	12/8/2018, 12/6/2018	0.48, 0.53 (0.36-84)
	(11/17/2018 -12/17/2018)	(0.38-82)
	(11/21/2018 -12/16/2018)	
C (cage free layers)	12/9/2018, 12/7/2018	0.5, 0.68 (0.34-2.35)
	(11/24/2018 -12/20/2018)	(0.36-2.3)
	(11/26/2018 -12/19/2018)	
D (caged layers)*	11/28/2018, 11/24/2018	0.33, 0.36 (0.26-0.58)
	(11/6/2018 -12/10/2018)	(0.28-0.55)
	(11/7/2018 -12/8/2018)	

*Results from barn D should be interpreted cautiously as the drop in egg production was mild to nonexistent, increasing the uncertainty in the estimated results.

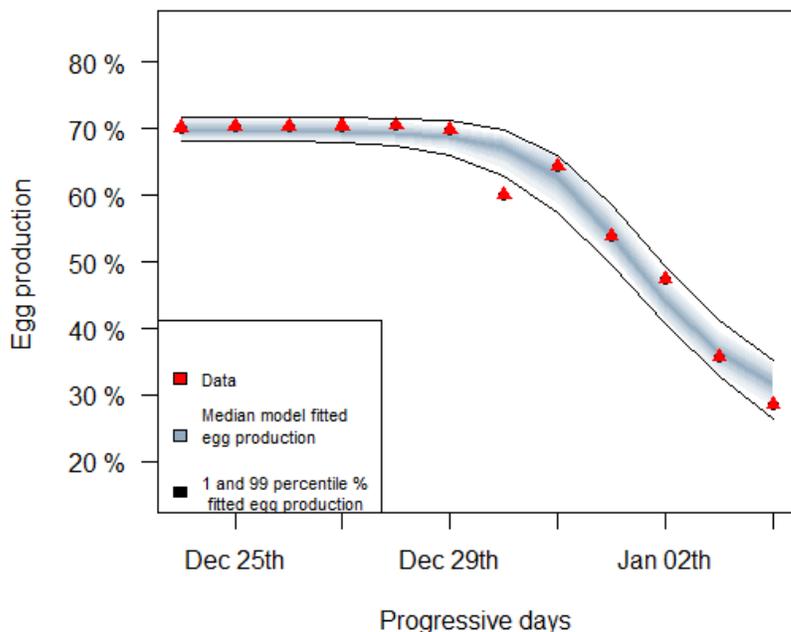


Figure D1: Model fitted egg production rate curves from the approximate Bayesian computation and observed egg production rate for Barn A. The shaded region represents the 95 percent credibility interval for the fitted egg production.

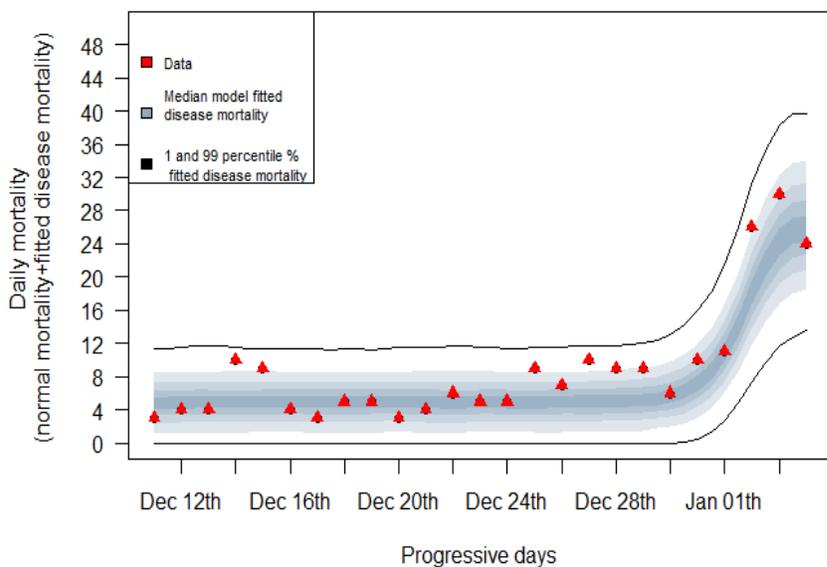


Figure D2. Model fitted disease plus normal mortality from the approximate Bayesian computation and the observed daily mortality for Barn A. The shaded region represents the 95 percent credibility interval for the fitted daily mortality.

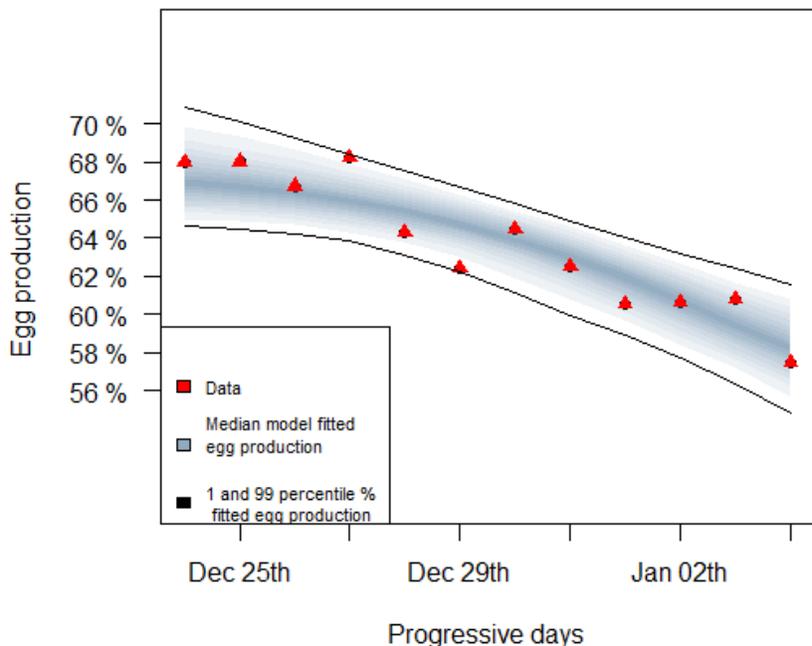


Figure D3. Model fitted egg production rate curves from the approximate Bayesian computation and observed egg production rate for Barn B. The shaded region represents the 95 percent credibility interval for the fitted egg production.

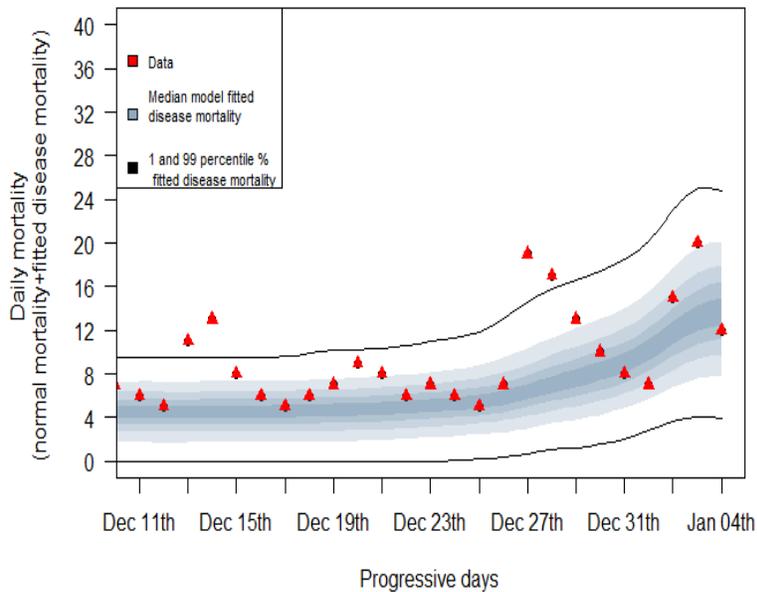


Figure D4. Model fitted disease plus normal mortality from the approximate Bayesian computation and the observed daily mortality for Barn B. The shaded region represents the 95 percent credibility interval for the fitted daily mortality.

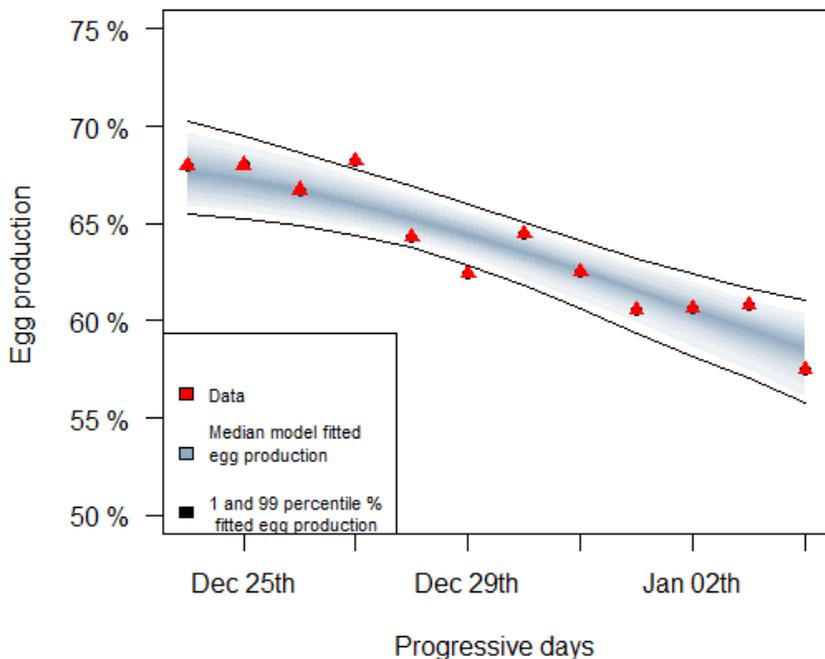


Figure D5: Model fitted egg production rate curves from the approximate Bayesian computation and observed egg production rate for Barn C. The shaded region represents the 95 percent credibility interval for the fitted egg production.

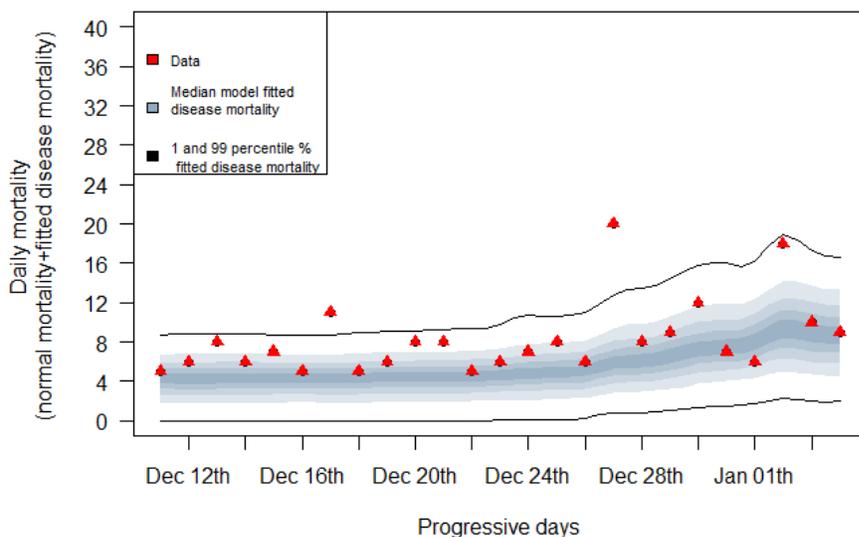


Figure D6. Model fitted disease plus normal mortality from the approximate Bayesian computation and the observed daily mortality for Barn C. The shaded region represents the 95 percent credibility interval for the fitted daily mortality.

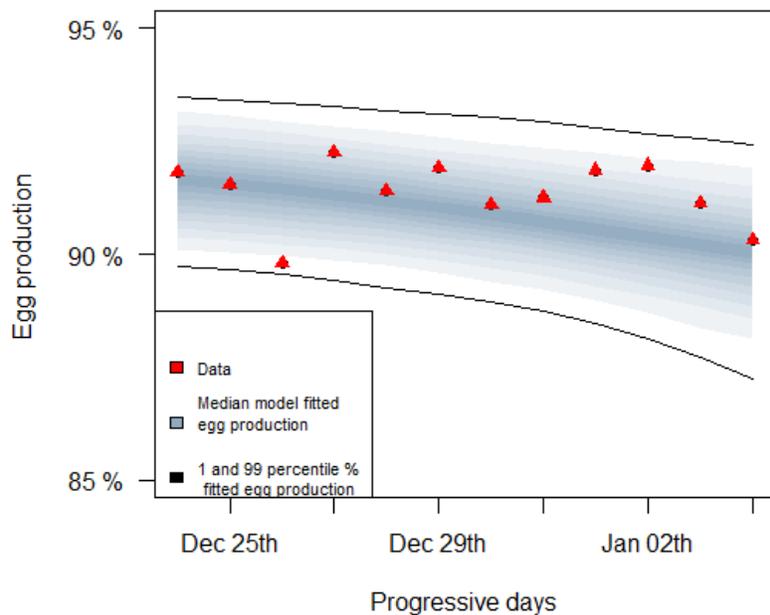


Figure D7: Model fitted egg production rate curves from the approximate Bayesian computation and observed egg production rate for Barn D. The shaded region represents the 95 percent credibility interval for the fitted egg production.

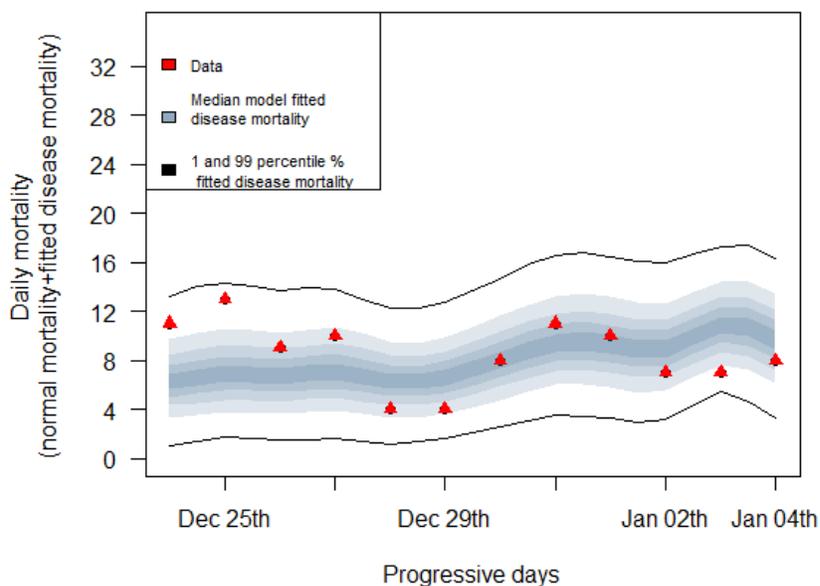


Figure D8. Model fitted disease plus normal mortality from the approximate Bayesian computation and the observed daily mortality for Barn D. The shaded region represents the 95 percent credibility interval for the fitted daily mortality.

**Epidemiologic Analyses of
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March 2021 Report

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