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THE NEED FOR FURTHER RESEARCH INTO THE TRANSMISSION OF INFLUENZA A VIRUSES BETWEEN MIGRATORY WATERFOWL AND DOMESTIC DUCKS

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Abstract: Migratory waterfowl are the natural reservoir and source point for influenza viruses. Artificial water ponds in rural and urban communities are potential sites of the human-animal interface for influenza A virus (IAV). This paper reviews the literature on the transmission of IAV between migratory waterfowl and domestic ducks in community ponds, and the potential for this to lead to human infection.
Keywords: Influenza A virus (IAV), Migratory waterfowl, Domestic ducks, Community ponds, Human infection, Transmission

Introduction and Background

Numerous research studies have indicated that migratory waterfowl are the natural reservoir and a source point for influenza viruses. Artificial water ponds in rural and urban communities are potential sites of the human-animal interface for IAV. Webster, Bean, Gorman, Chambers, and Kawaoka (1992) described this human-animal interface with the example of domestic ducks in community ponds attracting migratory waterfowl. The migratory waterfowl introduce influenza virus to that community's water pond from fecal contamination. The contaminated community water pond now becomes a potential source of influenza virus to both humans and animals. Influenza disease emergence data are collected year-round, but economic strain on global public health to prevent and treat human influenza outbreaks has been enormous. Therefore, it is imperative to identify potential sources of the virus to help minimize outbreak occurrence.

Statement of the Problem

There are gaps in knowledge about the association between molecular structure, epidemiologic and clinical characteristics, and the impact of ecological and other contextual aspects of IAV. Gaps in understanding the role of the physical and biogeochemical environment as an integral part of the IAV transmission also exist (Lang et al., 2008).More importantly, gaps in knowledge about the burden of IAV in rural and urban community settings remain present. The problem was that public health science professionals have been battling emerging human influenza diseases with tactile and reactionary methods because there was a lack of knowledge and data at the human-animal interface. The purpose of this baseline study of the proportion of IAV in urban and rural community settings was to provide knowledge and biological data of significant interest at the human-animal interface.

Emerging influenza viruses have continued to challenge public health officials. Influenza viral infections appear with such regular annual frequency that it has been common to refer to the phenomenon as *flu season* or *seasonal flu*. However, pandemic influenza events have not been regular or predictable. Taubenberger and Morens (2006) identified and provided a brief synopsis of 13 pandemic events between 1510 and 1978.Wali and Music (2011) provided a summary of public health outcomes of nine U.S. influenza epidemics that occurred between the period 1972 to 1973 through 1991 to 1992.Keeler (2011) provided a brief history of influenza pandemics from the time

of discovery of the influenza virus through the last decade. Further, Keeler notably identified the sharp increase in public awareness of influenza and the potential human health threat of the virus.The outcomes of this study and detection of IAV along the eastern Pacific flyway were in alignment with the World Health Organization (2011) description of the human-animal interface as a complex juncture at which new paradigms are emerging. Austin and Hinshaw (1984) suggested that surveillance of healthy ducks and the aquatic environment they frequent may be of significant interest to monitoring and controlling IAV. This research study is a model that can be modified to monitor IAV in the aquatic environment.

Purpose of the Study

In this study, the proportion and probability of the presence of IAV were investigated in recirculating artificial ponds in rural and urban geographical locations. Rural ponds were viewed as one population and urban ponds as another population. The dependent agent variables were IAV. The research question was as follows: Is there a difference in the burden of IAV in rural ponds compared to urban ponds?

Definition of Terms

Several not commonly used terms-outside of the topic of influenza-are necessary for this research study. The manuscript by Reid, Taubenberger, and Fanning (2004) includes definition of these terms. *Antigenic drift:* Minor changes in viral antigens due to gradual accumulation of mutations over time. *Antigenic shift:* Sudden change in viral antigens due to acquisition of one or more novel surface-protein-encoding genes by the process of reassortment. *M gene sequence:* The membrane protein (M) gene of IAV is 1,027 nucleotides long and encodes two proteins, M1 and M2. M1 protein is the most abundant protein in the influenza a virus viron (Webster et al., 1992). *Reassortment:* Due to the segmented nature of the IAV genome (eight individual RNA segments), influenza viruses can undergo a process of genetic reassortment to produce new variant strains of virus. In a cell infected with two different IAV strains, gene segments from each can be packaged into viable hybrid virus strains. *Subtype:* A designation for IAV describing the antigenic group to which the two dominant surface glycoprotein's — haemagglutinin (HA) and neuraminidase (NA) — belong, written in the form HXNX, wherein one of the 16 possible HAs and one of the nine possible NAs is listed, for example, H1N1 or H3N2. These terms are necessary and used throughout this study for clarity.

Assumptions and Limitations

There are several assumptions about IAV that are believed, but cannot be demonstrated to be true. Firstly, the unique and problematic property of IAV to evade hosts immune responses. Secondly, the phenomena of human-animal transmission are not well understood. It is possible the ability to evade host immune responses and the humananimal transmission are the evolutionary traits that give IAV a "natural fluid" presence resulting in seasonal outbreaks. Keeler (2011) and others asserted the emergence of novel IAV through antigenic drift or antigenic shift can result in significant increases in morbidity and mortality during any given influenza season. The outcome of antigenic drift or antigenic shift are routinely identified, however the mechanism of the processes are insofar only theorized. A pandemic is the outbreak of infection, arising in a specific geographical area and spreads throughout the world resulting in a high percentage of infected individuals and resulting in increased mortality rates (Potter, 2001).It has been assumed that pandemics may be caused by a new IAV subtypes. The new IAV subtype may have an HA of which is not related to that of influenza viruses circulating immediately before the outbreak, and could not have arisen from those viruses by mutation (Potter, 2001).The 1918 Spanish flu is assumed to be caused

by a "new IAV" subtype. Investigation into the influenza virus responsible for the 1918 Spanish flu has not yet been fully discovered. Investigation into the molecular mechanisms of IAV is prominent in current literature. This may be due to recent advances in molecular and genetic sciences. Artificial water ponds in rural and urban communities are potential sites of the human-animal interface. The investigation of the burden of IAV in artificial rural and urban ponds may be of significant interest to the scientific community. The intent of this study was to investigate the burden of IAV in artificial reticulating water ponds in the geographic locations of rural and urban Californian communities. The geographical sampling region for rural and urban ponds was the eastern Pacific flyway for migratory birds in California. For this study, it was necessary to make the assumption that artificial water ponds in rural and urban communities are sites of the human-animal interface because of fecal shedding and contamination from IAV infected waterfowl (*Anseriformes*) and shorebirds (*Charadriiformes*).The primary goal of the laboratory analysis was to isolate and investigate IAV in samples collected from water ponds in rural and urban Californian communities. There are two threats to the external validity of this study. The first threat to external validity is centered to the theoretical framework of this study. The theoretical framework for this study were based on the hypotheses proposed by Webster et al. (1992) and others that (a) migratory waterfowl are the natural reserve of influenza viruses, and (b) water-borne transmission of influenza virus occurs between migratory waterfowl and domestic waterfowl. The inclusion criteria of the study population were artificial recalculating water ponds in the geographic locations of rural and urban Californian communities. The geographical area was the state boundaries of California. Thus, the findings of this are limited to geographical locations along the migratory flyways of the waterfowl within the boundaries of California. To address this threat to the external validity of this study, the sampling and data analysis plan are presented in a fashion so the study may be reproduced accurately by others using the same or different geographical locations. The second threat to external validity is a result of the inclusion criteria. Recirculation artificial water ponds require mechanical equipment for recirculation. This study does not include the type or design of the mechanical equipment used for recirculation as an independent variable. Thus, the mechanical equipment used for recirculation cannot be evaluated as a mediator or moderator of the outcome variables of this study. However, estimated surface area of the recirculating artificial water ponds as an independent variable may be of significant interest to future studies seeking to investigate factors that associate to the burden of IAV at the human-animal interface in communities. The primary data collection of study is heavily weighted by laboratory instrumentation and molecular analysis. There are two threats to the internal validity of this study: instrumentation and laboratory assays. Firstly, inaccuracy of the instrument used for this study may systematically alter the data. Secondly, inaccuracy of the laboratory assays may fail to accurately detect IAV. To address the possible inaccuracy of the instrumentation, the same instruments were used on all samples. Thus, errors, if identified, may be corrected across all samples equally. To address the possible inaccuracy of the laboratory assays, positive and negative control samples were to be processed simultaneously as study samples. The positive control for IAV was not obtained. The negative control used for IAV was sterile normal saline. Threats to statistical conclusion validity have not been identified.

Literature Review

History of Influenza Surveillance

In 1947, at the 4th International Congress for Microbiology held in Copenhagen, a group of virologist forwarded a recommendation to the Interim Commission of the World Health Organization an international program be

initiated for influenza surveillance (Hampson, 1997). The World Health Organization Global Influenza Programme was established later that year (Fleming, van der Velden, & Paget, 2003). The 1947 influenza surveillance control programme is the oldest disease control program at the World Health Organization (World Health Organization, 2005). The World Health Organization stated the creation of the 1947 influenza surveillance control programme resulted from two concerns: first, the inevitable recurrence, at unpredictable intervals, of highly disruptive pandemics; and second, the significant health and economic impact of seasonal epidemics, which occur nearly every year (p. 34).Hampson (1997) opinioned the objectives of the 1947 influenza surveillance control programme as: to gain an understanding of the epidemiology of influenza, and to promptly isolate influenza viruses from new outbreaks and distribute them for vaccine production (p. S8).Within four years of creation, the 1947 influenza surveillance control programme developed into a network of 60 laboratories across 40 countries. The influenza surveillance control programme became the WHO Global Influenza Surveillance Network consisting of 113 national influenza centers located in 84 countries (World Health Organization, 2005).Following the adoption by the World Health Organization of the Pandemic Influenza Preparedness Framework in May 2011, the Global Influenza Surveillance Network was changed to Global Influenza Surveillance and Response System. The Centers for Disease Control and Prevention and the European Influenza Surveillance Scheme (EISS) collaborate with the WHO Global Influenza Surveillance and Response System to monitor the evolution of influenza viruses and provides recommendations in various disciplines including laboratory diagnostics, vaccines, antiviral susceptibility and risk assessment (World Health Organization, 2013).

Origin of Influenza (China)

The literature suggests China, with its abundance of live poultry and swine markets, maybe the epicenter of novel influenza viruses. Wan et al. (2005) proclaimed that Southern China has been shown to be the avian influenza virus pool for flu outbreaks in history including H2N2 (1957), H3N2 (1968), H5N1 (1997 & 2003), and H9N2 (1999).Webster et al. (1992) noted historical records and the appearance of the Asian, Hong Kong, and Russian pandemic strains of influenza virus in China suggest the majority of pandemics of human influenza since about 1850 have originated in China. Perez-Ramirez et al. (2011) described how large parts of Asia support high densities of humans, backyard poultry (ducks, geese, and chickens), pigs, and wild birds. These high density areas provide opportunities for close interaction between influenza reservoir animals and create a unique environment for influenza evolution (Perez-Ramirez et al., 2011).These unique environments may be considered an expansion of the mixing vessel theory; whereas these high density areas may be identified as “mixing vessel environments”.

Migratory Waterfowl (Vector for Global Distribution)

Austin and Hinshaw (1984) investigated feral duck species as a source of transmission of IAV and paramyxoviruses. The researchers collected swab samples from tracheal and cloacae from different species of feral ducks (Austin & Hinshaw, 1984). Austin and Hinshaw and were able to identify several viral strains of IAV. Additionally, Austin and Hinshaw asserted the alterations of influenza viral strains occur due to antigenic drift at point mutations and antigenic shift is caused by genetic reassortment.

The authors contended it was possible these alterations occurred in the intestinal tract of ducks (Austin & Hinshaw, 1984).This article provides support to the hypothesis that migratory ducks are a source point for influenza viruses. Therefore, surveillance of healthy ducks and the aquatic environment they frequent may be of significant interest to monitoring and controlling IAV. As previously stated, the literature suggests novel influenza

viruses originate in the Southern China region where migratory waterfowl become infected. Infected asymptomatic migratory waterfowl may transport the novel influenza viruses in their intestinal tracts to the wetland breeding grounds of Alaska and Siberia. The waterborne transmission of the influenza viruses to new hosts (migratory waterfowl from Northern and Southern American regions) may occur at these Alaskan and Siberian wetland breeding grounds. The new host(s) may act as a mixing vessel for influenza viruses. This may result in an antigenic shift or antigenic drift of influenza viruses resulting in novel strains. Infected asymptomatic migratory waterfowl may then transport these influenza virus strains along the eastern Pacific flyway for migratory birds. This process provides a possible explanation for the global distribution of novel influenza strains and further supports the necessity of influenza virus surveillance at the human-animal interface.

Water Analysis

Water analysis has been an important scientific approach to understanding the epidemiological triangle model of influenza viral diseases. In the early study by Cumming (1919), a case-control approach was used to better understand the transmission and infectivity of the causitive agent of influenza-pneumonia. The findings of Cumming study suggested the agent was the influenza-pneumonia organism, the route of transmission was contaminated eating utensils, and aquatic dishwashing was the environment. Webster et al. (1992) asserted waterborne transmission of influenza viruses occurs due to the viral shedding in the fecal material of waterfowl (*Anseriforms*) and shorebirds (*Charadriiformes*). Van Dalen et al. (2010) investigated waterborne transmission of influenza A H4N6 in mallards (*Anas platyyrhychos*) in a controlled laboratory environment. Lang et al. (2008) collected and analyzed sediment samples from three ponds in the Creamer's Field Migratory Waterfowl Refuge, Alaska, a location used by a wide variety of migratory waterfowl. The sediment samples were collected using a time-series approach and analyzed for IAV RNA using reverse transcription-polymerase chain reaction (RT-PCR) methodologies (Lang et al., 2008). Zhang et al. (2006) collected and analyzed samples of ice or water from three northeastern Siberian lakes in the Koluma River region. The samples were analyzed for the presence of IAV using reverse transcription-polymerase chain reaction (RTPCR) methodologies (Zhang et al., 2006).Hinshaw et al. (1980) collected samples from waterfowl, unconcentrated lake water, and feces from lake shores near Vermillion, Alberta, Canada. These samples were used to isolate influenza viruses and to investigate whether influenza viruses continually circulate or whether the same or different strains are present from year to year (Hinshaw et al., 1980).

Methodology

Research Design

The epidemiological triangle provided framework for the primary data collected.

The epidemiological triangle has three vertices: host, agent, and environment. The host is the organism harboring the disease. For influenza disease, the reservoir hosts are migratory waterfowl and shorebirds (Franklin et al., 2011).The agents are the IAV. The environments are the natural and artificial habitats where the hosts are found.

The independent environmental variables of this study were the geographical locations of the artificial water ponds in either a rural or urban community. The dependent agent variables were IAV.IAV data included detection for M gene sequence by real time RT-PCR using World Health Organization recommended primer sequences.

Sampling Methods and Procedures

The study method was quantitative using a cross-sectional design. A convenience sampling approach was used. The geographical area was the state boundaries of California. Equal sample sizes from rural and urban

communities were attempted. A representative sampling from each of the 21 counties considered rural areas, and 37 counties considered metropolitan and not rural in California by California Business and Professions Code Section 19986(l) were attempted. The inclusion criteria of the study population were artificial recirculation water ponds in the geographic locations of rural and urban communities.

Water Sampling (Pre analytical Phase)

Water samples were collected as previously published in the *U.S.EPA Field Sampling Guidance Document #1225*.A dip sampler for water collection was used.

A dip sampler is useful for situations where a sample is to be recovered from an outfall pipe or along a pond bank where direct access is limited. The long handle on such a device allows access from a discrete location so as not to disturb wildlife (U.S. Environmental Protection Agency, 1999).The water volume per sample was 200 ml. Other researchers have collected 200 ml volume water samples for influenza studies (Zhang et al., 2011).

Sample Preparation (Pre analytical Phase)

Sample integrity was maintained until received in the laboratory for processing. Sample identification was maintained throughout the laboratory testing. Samples were filtered using Millipore Durapore 0.22 µm filters to remove particulate matter and other suspended contaminates. The filtered sample were poured into 250 ml centrifuge tubes and concentrated at 10,000 rpm for 20 minutes at 4°C using a Sorvall SL-1500 Super-Lite centrifuge rotor. 100 µL of RNA later RNA Stabilizer reagent (QIAGEN N.V.) was added to 100 µL of the concentrated pond water sample in a separate polypropylene micro vial for viral RNA extraction. Evers et al. (2007) studied the commercial preservative RNA later (QIAGEN N.V.) by evaluating against the current method of cryo-freezing, and ethanol preservatives for IAV samples. From the findings of the Evers et al. study, the authors asserted the commercial preservative RNA later held at ambient temperatures might be useful for the identification of IAV in samples collected from infected waterfowl.

Real Time RT-PCR (Analytical Phase)

Reverse transcriptase polymerase chain reaction (RT-PCR) is a powerful technique for the identification of influenza virus genomes (World Health Organization, 2007). A constellation of studies have targeted the M gene sequence of the IAV genome by RT-PCR as an indicator of IAV positivity (Harmon et al. 2010; Lee et al., 2012; Magnard et al.,1999; Perez-Ramırez, et al., 2012).During the analytical phase of this study real time RT-PCR technique was utilized. Using real time RT-PCR allows for the detection of products as amplification is ongoing, allowing quantification (World Health Organization, 2007).The analytical phase was conducted following World Health Organization Real-time RT-PCR Protocol 2 for IAV (H5N1) detection.

Data Collection and Analysis

The primary data analysis was a cross-sectional approach comparing proportions recirculation artificial ponds in rural and urban geographical locations. The independent variable was: geographic community location (rural or urban). The dependent variable was: IAV detection by real time RT-PCR. The statistical analysis software was IBM SPSS Statistics 21 and Microsoft Office Excel 2007. Geographical graphing and mapping software was Google Earth. Data integrity was maintained by recording data and observations with ink into field notebook and laboratory notebook. Hand written entries were transferred or transcribed to Microsoft Office Excel 2007 Spreadsheets. All electronic data had digital backup and was password protected.

Credibility and Validity

Credibility is important to uphold society's trust that scientific research results are an honest and accurate reflection of a researcher's work (National Academy of Sciences, National Academy of Engineering, and Institute of Medicine of the National Academies, 2010). The materials and methods of this environmental baseline research study were built on the research of others. Environmental water sampling underscored the research design of this study. Therefore, the research adhered to the Code of Ethics and Standards of Practice for Environmental Professionals. The objectives of Environmental Professionals are to conduct their personal and professional lives and activities in an ethical manner (National Association of Environmental Professionals, n.d).

Findings

Evaluation of Research Data

Artificial pond water samples were collected from 14 of the 21 counties considered rural areas, and from 25 of the 37 counties considered metropolitan and not rural in California by California Business and Professions Code Section19986(l). Urban counties' pond water samples [N_{Urban} = 100] were collected from 25 of the 37 counties considered metropolitan and not. Rural counties' pond water samples [N_{Rural}=82] were collected from 14ofthe21 counties considered rural and not metropolitan. Pond water samples were verified for their content of IAV genome by real time RT-PCR targeting the M gene sequence. The real time RT-PCR M gene assay was conducted using a Bio-Rad CFX96 Touch Real time PCR Detection System (BioRad, Hercules, California) instrument and utilized two fluorescent dyes: FAM and HEX. The analysis software used was Bio-Rad CFX Manager 3.1. The software setting was: End cycles to average [5] and Percent of Range [10.0]. The results for the FAM fluorophore were: [Lowest RFU value = -3.71], [Highest RFU value = 3.41], [Negative Control Average = -0.453], and [Cut Off Value = -0.0668]. The number of statewide samples [N = 182] called (+) positive using FAM [N_{FAM} = 45, 45/182 = 24.7%].The results for the HEX fluorophore were: [Lowest RFU value = -1.93], [Highest RFU value = 1.46], [Negative Control Average = 1.42], and [Cut Off Value = 1.42].The number of statewide samples [N = 182] called (+) positive using HEX [N_{HEX} = 1, 1/182 = 0.5%].The number of statewide samples [N = 182] verified for their content of IAV genome by real time RT-PCR targeting the M gene sequence is [n = 45] (see Figure 1.).The confidence interval formula for a proportion is commonly known as: $CI = p \pm Z_{\alpha/2} \times \sqrt{[(p \times q) / n]}$, $(x, n - x \geq 5)$, where $p = x / n$, $q = 1 - p$, $\alpha = 1 - (\text{Confidence Level}/100)$ x = Frequency, n = Sample Size, and $Z_{\alpha/2}$ = Z-table value. Applying the data values of this study into the confidence interval formula the proportion of the overall statewide samples [N = 182] called (+) positive for the IAV M gene sequence [n = 45] is $P = 0.247$, 95% CI [0.185, 0.310].The effect size index Cohen's g is the departure from $P = 0.50$ (Cohen, 1988).Thus, the non directional calculation for Cohen's g is [$g = |P - 0.50|$].For the overall statewide samples, the calculation of Cohen's g is [$g = |0.247 - 0.50| = 0.253$]; a large effect size (Cohen, 1988). Using G*Power 3.1.5, the post hoc computed Power = 0.999 (two tailed).

Urban samples assayed by real time RT-PCR

The number of urban county samples [N_{Urban} = 100] called (+) positive using FAM [N_{FAM} = 36, 36/100 = 36.0%].The number of samples [N_{Urban} = 100] called (+) positive using HEX [N_{HEX} = 1, 1/100 = 1.0%].The number of urban county samples [N_{Urban} = 100] verified for their content of IAV genome by real time RT-PCR targeting the M gene sequence is [n = 36].Applying the confidence interval formula for a proportion: $CI = p \pm Z_{\alpha/2} \times \sqrt{[(p \times q) / n]}$, $(x, n - x \geq 5)$, where $p = x / n$, $q = 1 - p$, $\alpha = 1 - (\text{Confidence Level}/100)$ x = Frequency, n =

Sample Size, and Z $_{\alpha/2}$ = Z-table value, the proportion of the urban counties' samples [N_{Urban} = 100] called (+) positive for the IAV M gene sequence [n = 36] is *P* = 0.36, 95% CI [0.266, 0.454]. The effect size index Cohen's g is the departure from *P* = 0.50 (Cohen, 1988).Thus, the non directional calculation for Cohen's g is [g = |*P* – 0.50|].For the urban counties' samples, the calculation of Cohen's g is [g = |0.36 – 0.50| = 0.14]; a medium effect size (Cohen, 1988).Using G*Power 3.1.5, the post hoc computed Power = 0.768 (two tailed)

Rural samples assayed by real time RT-PCR

The number of rural counties' samples [N_{Rural} = 82] called (+) positive using FAM [N_{FAM} = 7, 7/82 = 8.5%].The number of samples [N_{Rural} = 82] called (+) positive using HEX [N_{HEX} = 2, 2/82 = 2.4%].The number of rural counties' samples [N_{Rural} = 82] verified for their content of IAV genome by real time RT-PCR targeting the M gene sequence is [n = 9].Applying the confidence interval formula for a proportions: CI = p ± Z $_{\alpha/2}$ × √[(p × q) / n] , (x, n – x ≥ 5), where p = x / n, q = 1 - p, α = 1 - (Confidence Level/100) x = Frequency, n = Sample Size, and Z $_{\alpha/2}$ = Ztable value, the proportion of the rural counties' samples [N_{Rural} = 82] called (+) positive for the IAV M gene sequence [n = 9] is *P* = 0.11, 95% CI [0.042, 0.177]. The effect size index Cohen's g is the departure from *P* = 0.50 (Cohen, 1988).Thus, the non directional calculation for Cohen's g is [g = |*P* – 0.50|].For the rural counties' samples, the calculation of Cohen's g is [g = |0.11 – 0.50| = 0.39]; a large effect size (Cohen, 1988).Using G*Power 3.1.5, the post hoc computed Power = 1.000 (two tailed).

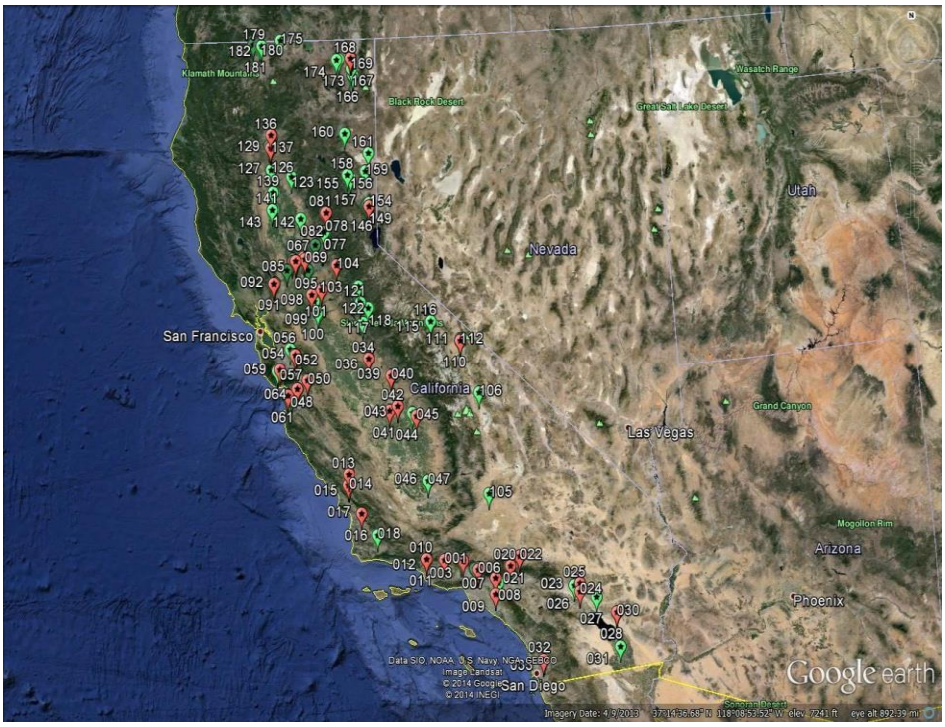

Figure 1: Statewide samples verified for their content of IAV genome. Google Earth map of California showing statewide pond water samples verified for their content of IAV genome by real time RT-PCR targeting the M gene sequence. [RED icon = (+) Positive for M gene sequence and GREEN icon = (-) Negative for M gene sequence].

Analysis of Relevant Research Data

In this study the proportion and probability of the presence of IAV was investigated in recirculation artificial ponds in rural and urban geographical locations. Rural ponds were viewed as one population and urban ponds as another population. The dependent agent variables are IAV.IAV data included: detection for M gene by real time RT-PCR using World Health Organization recommended primer sequences.

Examination of the Research Question

The proportion of the urban counties' samples [$N_{Urban} = 100$] called (+) positive for the IAV M gene sequence [$n_u = 36$] is $P_u = 0.36$, 95% CI [0.266, 0.454]. This value is numerically larger than the proportion of the rural counties' samples [$N_{Rural} = 82$] called (+) positive for the IAV M gene sequence [$n_r = 9$] is $P_r = 0.11$, 95% CI [0.042, 0.177].It is commonly accepted the two-sided 100 (1 - α) % CI for the difference between two proportions of unequal sample size is of the form: $(\hat{p}_1 - \hat{p}_2) \pm z_{1-\alpha/2} \sqrt{[(\hat{p}_1 (1 - \hat{p}_1) / n_1) + (\hat{p}_2 (1 - \hat{p}_2) / n_2)]}$. For this analysis; [$\hat{p}_1 = n_u / N_{Urban}$], [$\hat{p}_2 = n_r / N_{Rural}$], and [$z_{1-\alpha/2} = 1.96$].The calculated difference in the proportion of urban counties' samples called (+) positive for the IAV M gene sequence to rural counties' samples called (+) positive for the IAV M gene sequence is directionally towards urban counties' samples [$(\hat{p}_1 - \hat{p}_2 = 0.2502$, 95% CI (0.1278, 0.3604), *RD* = 0.2502, 95% CI (0.1337, 0.3668), *RR* = 3.28, 95% CI (1.83, 7.79), *OR* = 4.563, 95% CI (2.042, 10.193)]. The effect size index of the difference between two proportions is Cohen's h. Cohen's h is the calculated difference between the arcsine transformations of the proportions (*P*) of the populations (Cohen, 1988).Thus, Cohen's $h = |\varphi_1 - \varphi_2|$ where $\varphi = 2 \arcsin \sqrt{(P)}$.The calculated effect size index for the difference in the proportion of urban counties' samples to rural counties' samples called (+) positive for the IAV M gene sequence is Cohen's $h = |(2 \arcsin \sqrt{(P_u)}) - (2 \arcsin \sqrt{(P_r)})| = |(2 \arcsin \sqrt{(0.36)}) - (2 \arcsin \sqrt{(0.11)})| = |1.287 - 0.676| = 0.611$; a large effect size (Cohen, 1988).Using G*Power 3.1.5, the post hoc computed Power = 0.979 (two tailed) and actual α = 0.038.

Summary and Implications

Summary

This research study has been an investigation into the proportion of IAV in artificial suburban neighborhood water ponds. No known research has analyzed the proportion and persistence of influenza viruses in these aquatic habitats. To investigate the proportion of IAV in recirculation artificial ponds, 182 pond water samples were collected from a representative sampling from 14 counties considered rural areas ($N_{Rural} = 82$), and 25 counties considered metropolitan and not rural ($N_{Urban} = 100$) in California by California Business and Professions Code Section 19986(l) was achieved. Field research data and laboratory data were transcribed to a Microsoft Office Excel 2007 spreadsheet and statistically analyzed to answer the research question of this study. The analysis of the proportion of IAV in rural and urban water ponds favored the greater burden of IAV in urban community ponds over rural community ponds [$(\hat{p}_1 - \hat{p}_2 = 0.2502$, 95% CI (0.1278, 0.3604), Cohen's h = 0.611, Power = 0.979, actual α = 0.038].

Implications

The potential impact of this study can be recognized at the international level, the national and state level, the population level, and at the individual level. The results show that artificial water ponds in communities can be sources of IAV.It is commonly known that IAV can result in a zoonotic disease. Infectivity and sub typing of isolated IAV may identify strains previously known capable for animal-human transmission, or human-human

transmission.As sources of IAV, the artificial water ponds in communities can be used to increase IAV surveillance and monitoring at the human-animal interface, expand and improve upon the IAV strain library for vaccine development, and may bring greater awareness to the individual, and thus, greater perceived susceptibility to IAV infection leading to improved vaccination rates. International surveillance and monitoring of influenza viruses is a current global challenge.

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