# The Burden of Avian Influenza Viruses in Community Ponds in California

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## Abstract

This research was a baseline study of the proportion of influenza A virus (IAV) in urban and rural communities in California. The population was artificial recirculating water ponds in the geographic locations of rural and urban Californian communities. Surface water samples were collected from artificial recirculating ponds in California.

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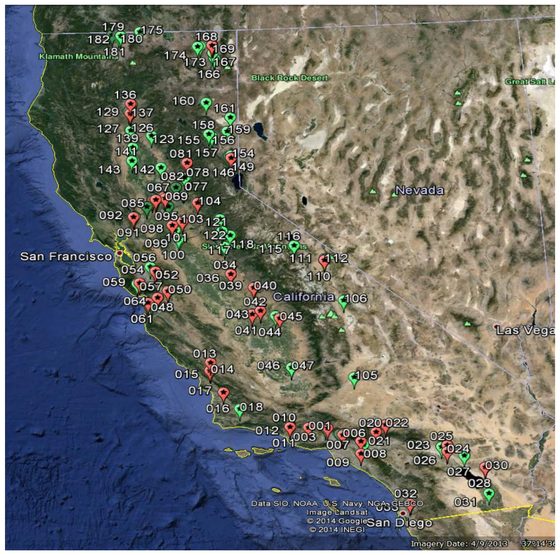
## Problem

The problem is 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. 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 influenza viruses.

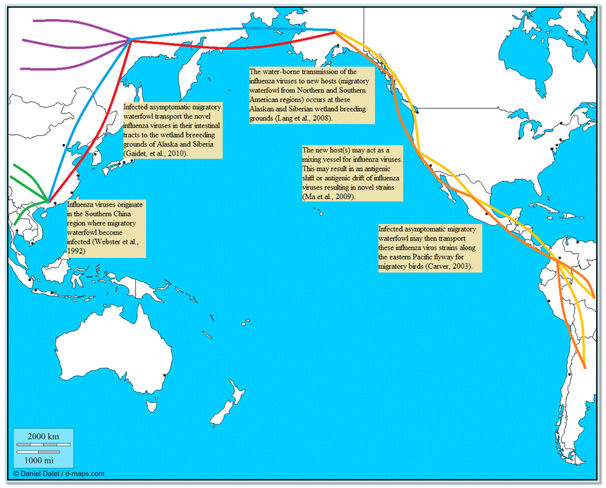
## Purpose

The purpose of this quantitative study was to extend the previous environmental virology research of influenza in natural waterfowl wetlands to rural and urban communities. The role of the aquatic virus reservoir as a human-animal interface has not been fully understood. This dissertation study was focused towards investigating the aquatic virus reservoir.




## Relevant Literature

Aquatic virus reservoirs is a human-animal interface. Franklin et al. (2011) suggested aquatic virus reservoirs give rise to indirect transmission, which would alter the transmission dynamics, beyond just direct interactions between infectious and susceptible individuals. The literature suggests novel influenza viruses originate in the Southern China region where migratory waterfowl become infected (Webster et al., 1992).




## Research Questions

*(R1)*: Is there a difference in the burden of influenza A virus in rural ponds compared to urban ponds?
*(R2)*: Is there an association between geographic location, altitude, estimated surface area and observed presence of waterfowl, and the proportion of influenza A virus in rural ponds compared to urban ponds?
*(R3)*: Is there an association between water pH, salinity, or water temperature and influenza A virus infectivity in rural and urban water ponds?
*(R4)*: Are H5N1 influenza A viral subtypes detected in the sample of rural and urban ponds novel gene sequences or have the gene sequences been previously identified elsewhere?

## Procedures

A representative sampling from each of the 21 counties (rural areas), and 37 counties (metropolitan) in California were attempted. Surface water samples were collected from artificial recirculating ponds. IAV(+) was verified by real-time RT-PCR, MDCK cells for virus infectivity, nucleotide sequencing of the RNA genome, and phylogenic analysis of IAV H5N1 strains. pH, salinity, and temperature of water samples were analyzed. Pond surface areas were calculated using a laser rangefinder, GARMIN® GPS, or Google Earth.

## Data Analysis

Included proportions, bivariate and multivariate logistic regression analysis. The IVs included: geographic community location (rural or urban), GPS location (latitude and longitude), altitude, approximate water pond surface area. The DVs included: IAV detection, IAV infectivity endpoint titer, IAV (H5N1) characterization. Variables evaluated as possible mediators and moderators included: water pH at collection, water salinity at collection, water temperature at collection, and presence or absence of waterfowl (*Anseriformes*) and shorebirds (*Charadriiformes*) at collection.

## Findings

Showed an association in the burden of influenza A virus to geographical location. The data favored a greater burden of IAV in urban ponds compared to rural ponds. The data analysis—MDCK plaque assays, real time RT-PCR, and nucleotide sequencing—supported the proposal by Franklin et al. (2011) that aquatic systems may serve as reservoirs and sources of infection for both wild birds and mammals. Thus, the aquatic habitats sampled for this study were potential sites of the human-animal interface of IAV. Interestingly, the analysis of the data did not fully support the well-accepted theory of the influenza virus human-animal interface by Webster et al. (1992).

## Limitations

The findings are limited to geographical locations along the migratory flyways of the waterfowl within the boundaries of California. Severe drought conditions limited available water ponds for sampling. Summer 2014 wildfires in N. California made several water locations unsafe for travel and field research. Unsafe smoke and fire across regions are likely to have diverted migratory waterfowl migration towards safer flight paths and stopovers.

## Conclusions

Community water ponds are viable sites of IAV. Data analysis suggest aquatic systems are reservoirs and sources of infection for wild birds and mammals. Infectivity studies show community water ponds to be sites of the human-animal interface for IAV infection. This environmental baseline study validated community water ponds as resource sites for IAV surveillance and monitoring. The commercial preservative RNAlater (Qiagen®) was successfully used as a stabilizing media for IAV research. Real time RT-PCR utilizing the TaqMan® methodology was successful. Field water samples with the MDCK plaque assay showed promise and warrants further investigation. Geochemical properties of pond water was not concluded as contributing to IAV infectivity in water ponds. MDCK plaque assay to geochemical properties of pond water as contributing factors to IAV infectivity warrants further investigation.

## Social Change Implications

Impact of this study extends to the international level, the national and state level, the population level, and at the individual level. The results of this study show artificial water ponds in communities can be sources of IAV. Artificial water ponds in communities can be used for IAV surveillance and monitoring, expand and improve upon the IAV strain library for vaccine development, and may bring greater awareness to the individual, and thus, leading to improved vaccination rates.

*Dr. Richard Jimenez, Chair; Dr. LaToya Johnson, Member; Dr. Rodney Bowden, URR.*

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