

# Evaluating the Impact of Dairy Processor Inputs on Avian Influenza A (H5) RNA Levels in California Wastewater

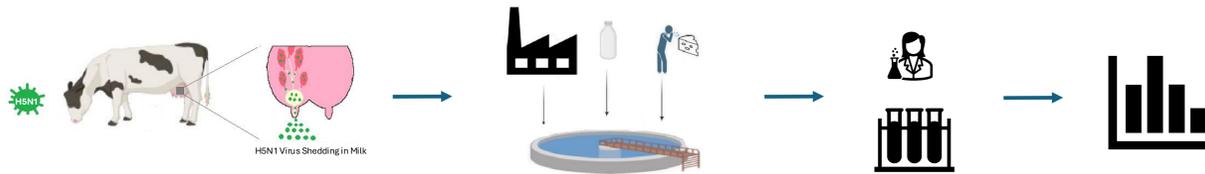
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## Background:



- H5 viral RNA in wastewater can originate from human and non-human sources via animal products (e.g., dairy) and animal feces.
- H5 concentrations are high in milk due to the H5N1 virus localizing in the mammary glands of infected cattle.
- FDA studies show H5 RNA is detectable in dairy products even after pasteurization.
- Dairy farms are not permitted to discharge animal waste to municipal sewer systems.
- Large volume dairy processing facilities are permitted to discharge wastewater into municipal sewer systems (e.g., waste from byproducts, industrial applications, and cleaning equipment).

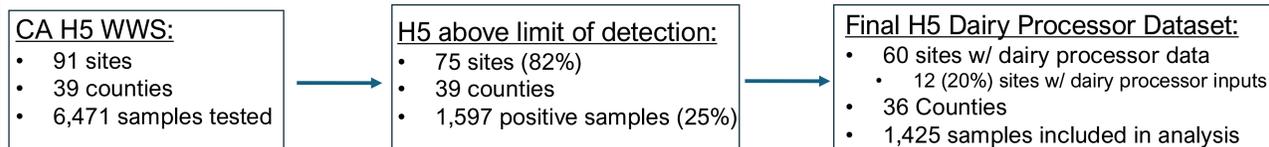


**Hypothesis:** H5 wastewater concentrations will be higher in California sewersheds with inputs from dairy processor(s).

**Objective:** Evaluate the relationship between H5 RNA concentrations in California wastewater samples and the presence or absence of dairy processor inputs.

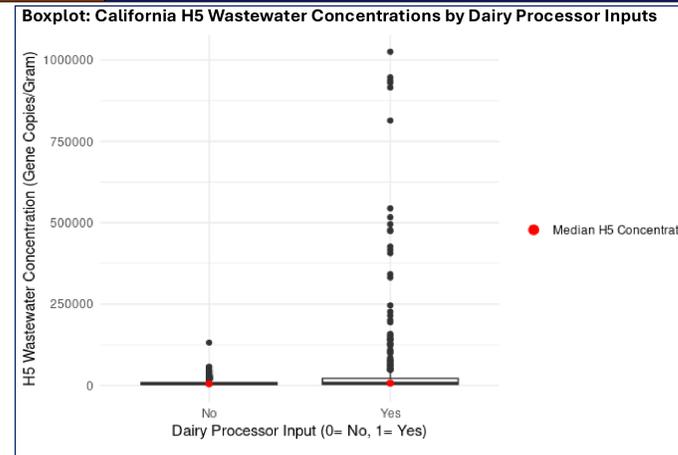
## Methods:

- Study period:** May 15, 2024 – January 31, 2025
- Data:**
  - Wastewater data obtained via three programs:
    - WastewaterSCAN
    - CDPH's Drinking Water and Radiation Laboratory (DWRL)
    - CDC's National Wastewater Surveillance System (NWSS)
  - Dairy processor input data collected from utility partners after initial H5 detection
  - Health officer regions:
    - Bay Area (ABAHO), San Joaquin Valley (SJCVC), Greater Sacramento (SAC), Rural North California (RANCHO), Southern California (SoCal)
- Data Inclusion:**



- Testing Method:**
  - Digital droplet PCR
  - PCR Gene Target: Influenza A H5 Hemagglutinin (HA)
  - H5 concentrations: gene copies/gram of wastewater or liter of wastewater (varies by laboratory)
- Analysis:**
  - Linear mixed-effects model
    - Statistical analysis via R (version 4.3.2) utilizing LME function
  - Measure association between H5 RNA concentrations ( $\log_{10}$ -transformed) and presence of dairy processor inputs
    - $\text{Log}_{10} \text{ H5 Conc} \sim \text{Dairy Input} + \text{Region} + \text{Lab (Fixed Effects)} | \text{WWTP Site (Random Intercept)}$

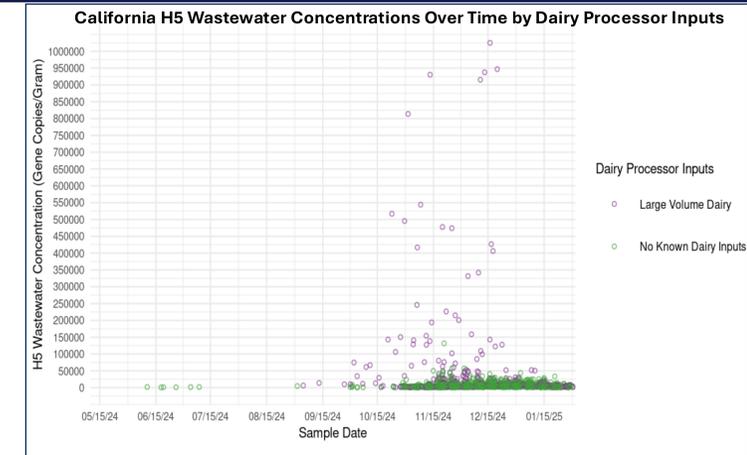
## Results (May 15, 2024 – January 31, 2025):



Dairy Processor Input	Median H5 Concentration (gene copies/gram)	H5 Concentration Range (gene copies/gram)
Yes	7,063.74	732.60 - 1,025,123.00
No	4,938.91	758.48 - 131,443.10

**Table 1:** Summary of boxplot results comparing H5 concentrations for California wastewater surveillance sites with and without dairy processor inputs.

In the linear mixed-effects model, the presence of a dairy processor input was significantly associated with a mean 1.61-fold ( $\beta = 0.205$ ,  $p = 0.006$ ) increase in wastewater H5 RNA concentrations compared to sites without processors present, while adjusting for regional differences, laboratory data source, and accounting for site-level variation.



Predictor	Estimate (Back-Transformed)	Std. Error	95 % CI (Lower-Upper)
<b>Intercept</b>	3526.20	0.05	<b>3180.60 - 3908.20</b>
<b>Dairy Input: Yes</b>	1.61	0.07	<b>2.38 - 1.86</b>
<b>Region: RANCHO</b>	1.80	0.12	<b>1.02 - 3.16</b>
Region: SAC	1.41	0.09	0.99 - 1.98
<b>Region: SJCVC</b>	1.86	0.10	<b>1.26 - 2.76</b>
<b>Region: SoCal</b>	1.56	0.07	<b>1.39 - 1.86</b>
<b>Data Source: DWRL</b>	1.72	0.05	<b>1.56 - 1.89</b>
<b>Data Source: CDPH NWSS (Verily)</b>	1.94	0.10	<b>1.31 - 2.86</b>
Data Source: WastewaterSCAN	1.19	0.05	0.99 - 1.43

**Table 2:** Summary of results from linear mixed-effects regression model with a random intercept for wastewater treatment plant site. Estimates are back-transformed ( $10^{\beta}$  estimate) and reflect difference relative to the reference groups. Bolded values indicate statistical significance at  $p < 0.05$ . Reference categories: Region = ABAHO; Data Source = CDC NWSS Commercial Contract (Verily); Dairy Processor = No.

## Conclusion:

- These results support our hypothesis that discharge of wastewater and dairy byproducts from dairy processing facilities significantly impact H5 concentration levels.
- The stark differences in upper concentration ranges highlight the potential to use wastewater concentration data to identify potential sources of H5 in wastewater, including contributions from local industrial sources.
- Understanding these patterns can improve the interpretation and utility of H5 wastewater concentration data (i.e. Provide context to H5 signals driving total influenza A levels) for public health and one health response (i.e. Act as an early warning system for changes to herd infections).

## Acknowledgments:

- Cal-SuWers Wastewater Utility Partners
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- CDC National Wastewater Surveillance System (NWSS)
- CDPH Drinking Water and Radiation Laboratory (DWRL)
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The findings and conclusions in this presentation are those of the author(s) and do not necessarily represent the views or opinions of the California Department of Public Health or the California Health and Human Services Agency.