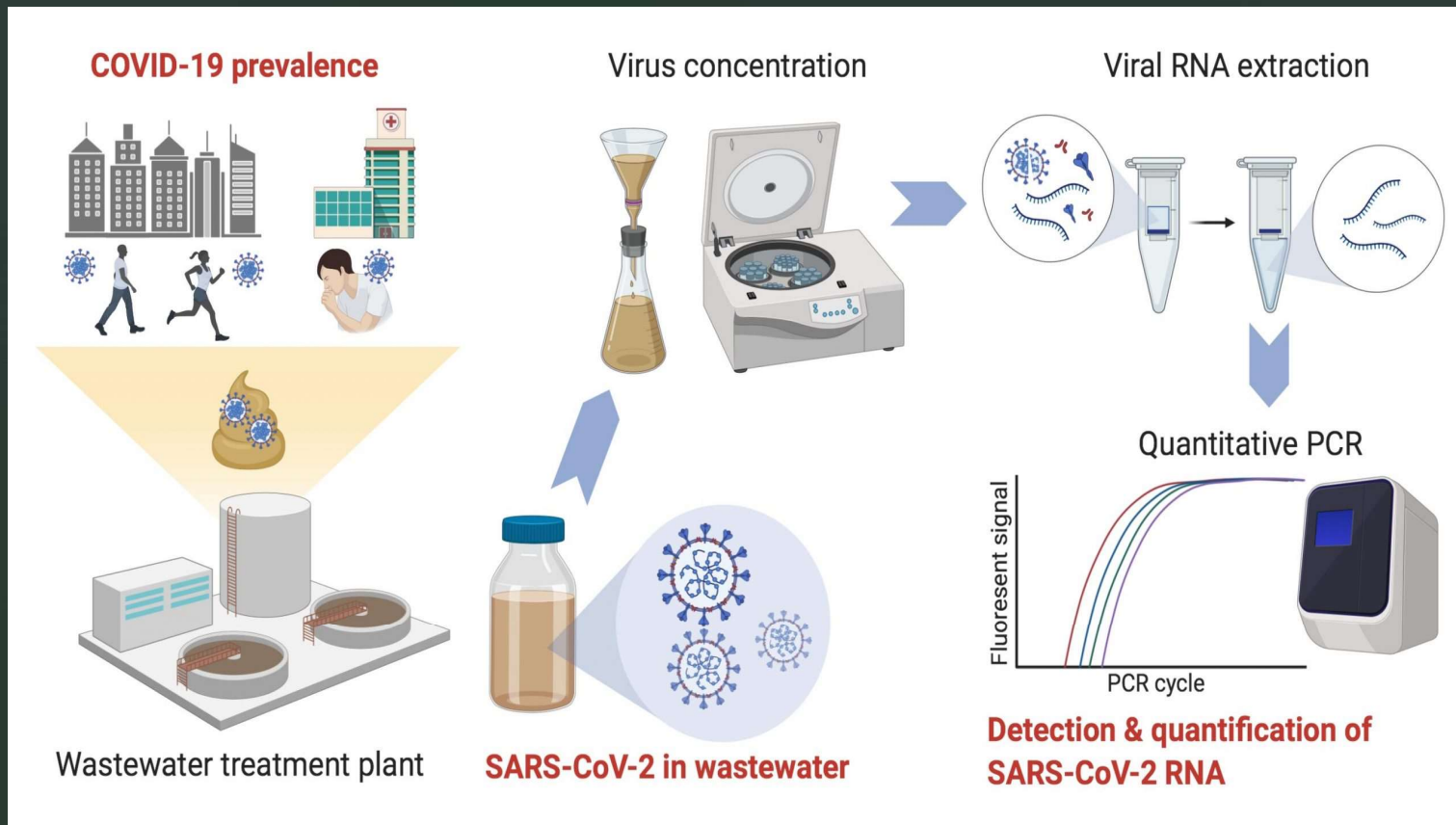


Quantification of SARS-CoV-2 Shedding Rate and % Asymptomatic Cases

Sarah Prasek, Bradley Schmitz, Ian Pepper



WASTEWATER-BASED EPIDEMIOLOGY



WASTEWATER-BASED EPIDEMIOLOGY (WBE)

- Rapidly growing discipline formerly known as “sewage surveillance”
- Relies on testing wastewater from a given community for the presence of a particular virus
- Answers the questions: “Is the virus in that community?” “Is there a pandemic?”
- Concentration of the virus gives an indication of the severity of the pandemic

WBE: CONCEPT FOR CORONAVIRUS

- Infected individual shed the virus in feces which then enters wastewater (sewage)
- Shedding occurs up to 4 days prior to visible symptoms
- Shedding at a maximum at onset of symptoms
- Shedding continues 2-4 weeks after disappearance of symptoms

BENEFITS OF WBE

- One test monitors defined community such as wastewater treatment plant service area or student dorm
- Highly sensitive: can detect 1 infection in 10,000 individuals
- Gives total virus load shed into wastewater including virus from symptomatic and asymptomatic individuals
- Is a leading indicator – can be detected in sewage 4-7 days prior to symptoms developing

ADDITIONAL BENEFITS OF WBE

- Useful for detecting onset of a pandemic
- *Quantitative* data over time determines if pandemic is \uparrow or \downarrow
- Allows determination of whether or not a community could/should return to work
- Can be used to evaluate the success (or lack thereof) of interventions such as mandated mask usage or social distancing
- Correlation of virus wastewater concentration (gene copies) with the number of clinical cases allows for future predictions of #s of infections

WEST CENTER MONITORING OF COVID IN SEWAGE FROM WASTEWATER TREATMENT PLANTS

- WEST WEBSITE (March 2020): offer to test samples nationwide for a fee
- March → August 2020, over 300 samples analysed
- Samples from all over U.S. including Los Angeles, New York, Seattle Jacksonville (FL)
- Raw wastewater samples often +ve
- Always –ve after 2° treatment and disinfection

META DATA

- Basic collection data: date, time, location
- Type of sample: raw sewage or after treatment
- WWTP service area
- Number of individuals served
- Number of cases in service area (on that date)
- Number of deaths (on that date)
- Look for correlations with virus concentration in wastewater

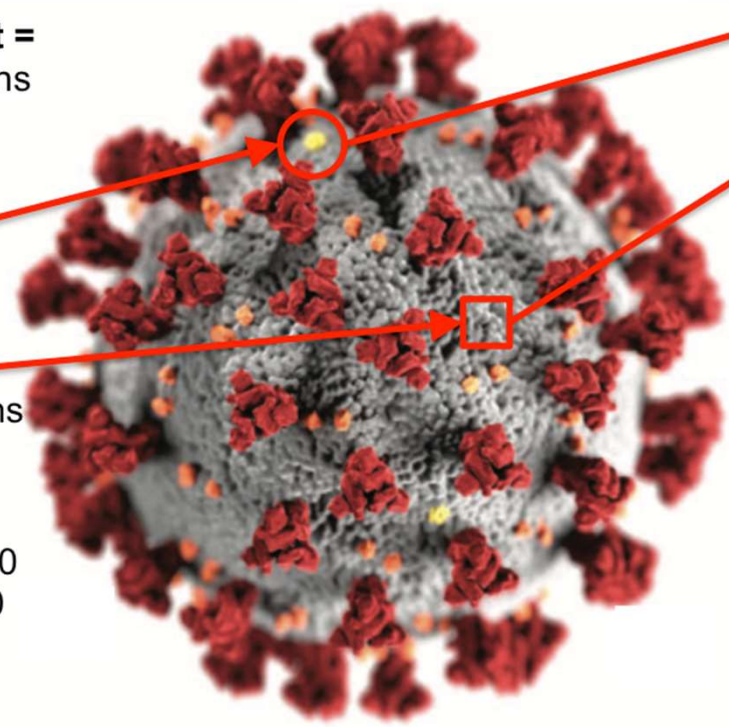


Chinese CDC Target =
Non-structural Proteins
(orf1a)

WHO Target =
Envelope Protein
(E)

CDC Target =
Nucleocapsid Proteins
(N1, N2, N3)

Sources: CDC, 2020
Corman et al., 2020
Lu et al., 2020



**SARS-CoV-2
Viral Structure**

The genome provides the genetic template for virus structures and proteins. We look for portions of the genome as an indication that the virus is present.

SENSITIVITY OF WBE: AGUA NUEVA WWTP

- “Stay at home” order in Arizona
 - Approximately 2-4 weeks later, virus concentrations and case count decrease
- “Re-open economy” order in Arizona
 - Approximately 7 days later virus concentrations increase
 - Approximately 2 weeks later, case count increases
- Memorial Day, Independence Day, Labor Day, Halloween, Thanksgiving, Christmas
 - Approximately 1 week after each holiday virus concentrations increase
 - Approximately 2 weeks after each holiday case count increases
 - Superbowl?

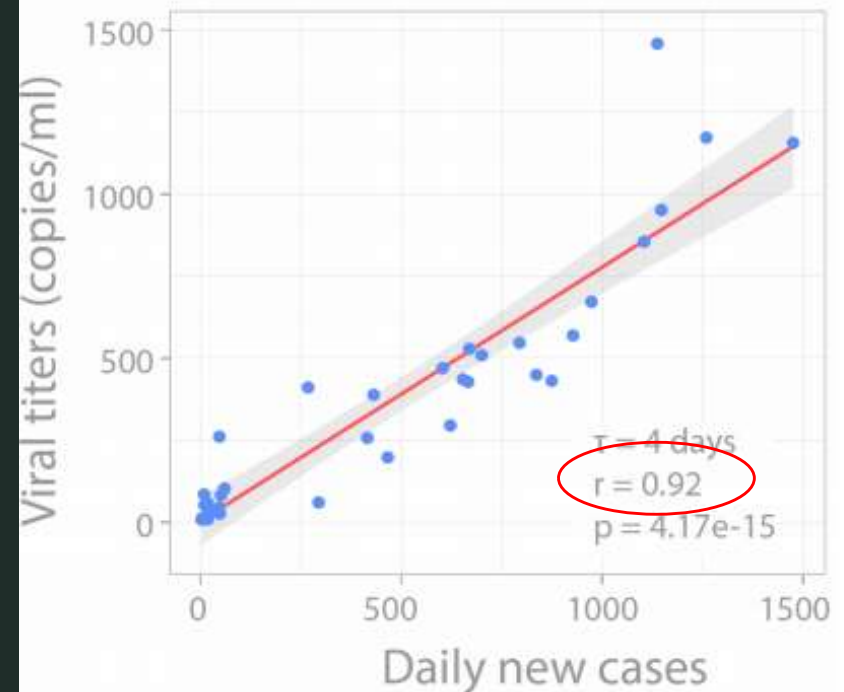
Pima County Data Modeling Efforts

Correlations

LOUDOUN WATER

Compare		tau	p	z
conc	model	0.898933	0.000328	3.5921
conc	infection	0.6	0.01667	36
model	infection	0.595437	0.007348	2.6806
conc	new	0.466667	0.07255	33
model	new	0.473296	0.03311	2.1308

- Modeled data is synonymous with observed data 👍
- Modeled data positively correlates with total infections 👍
- Model data positively correlates with new cases 👍
 - Observed RT-qPCR does not correlate with new cases, modeled data is more 'accurate'



SPRING 2020

- Pandemic begins
- Spring Break (March) University of Arizona shuts down
- Students leave
- All classes online
- **Costs UA \approx \$100m**

UNIVERSITY OF ARIZONA CAMPUS RE-ENTRY PLANS FOR FALL 2020

- 7 teams established June 2020
- COVID-19 testing of humans (RT PCR)
- Antibody testing (Elisa IgG Antibody Test)
- Contact tracing (In person and app. Based)
- Isolation (segregated dorms or hotels)
- Health Data Management and Communication (HIPAA and FERPA compliant data management)
- Thermometry (temperature measurement of individuals)
- **WBE: US! (Dormitory testing for early detection of in-house infections)**

DORMS TESTED

- Maricopa
- Kaibab/Huachuca/Arizona
- Sonora
- Arbol de la Vita
- Graham/Greenlee
- La Paz
- La Cienega
- San Pedro
- Santa Cruz
- **LIKINS**

Collecting Wastewater from Dorms



UNIVERSITY OF ARIZONA CAMPUS RE-ENTRY PLANS FOR FALL SEMESTER 2020

- 7 teams met and planned from June → August
- Discussions on how often to clinically test students
- Discussions on how often to test dorm wastewater
- Discussions on how to respond to a positive wastewater result

FALL CAMPUS RE-ENTRY BEGINS AUGUST 2020

- **Aug 18-24** Students begin returning
- **Aug 24** Fall semester begins
- **Aug 25** Wastewater positive from Likins Hall

All hell breaks loose

- **Aug 25**
 - 6:00pm results reported
 - 11:00pm Dr. Pepper awoken by President Robbins phone call
- Decisions made to retest wastewater and clinically test all students for COVID-19

- **Aug 26**
 - 5 wastewater samples collected – all positive
 - Clinical COVID tests identify two asymptomatic, but infected students

HOW WBE REDUCED EXPONENTIAL SPREAD OF COVID-19

- The two infected students were asymptomatic
- Without WBE detection and isolation, they would have spread COVID-19 to other students
- This scenario has been repeated ≈ 80 times
- University has successfully remained open
- Influence of “Shelter in Place” reflected in wastewater virus concentrations

OVERALL STORY RESULTED IN MEDIA FRENZY!

- Broadcasts with CNN, NPR, CBS, NBC, ABC
- Publications in “The Atlantic” and “Politico”
- 300 media hits in September

NATIONAL HEADLINES

The University of Arizona says it caught a dorm's covid-19 outbreak before it started. Its secret weapon: Poop.

How the University of Arizona used No. 2 to solve its No. 1 problem: The coronavirus

University of Arizona's wastewater testing halts potential surge in COVID-19 cases at dorm

University of Arizona wastewater testing finds virus at dorm, prevents outbreak

Wastewater helps find positive COVID-19 cases at UA dorm

Poop tests stop COVID-19 outbreak at University of Arizona

UA wastewater testing finds COVID-19 cases in dorm

Researchers at the University of Arizona say they stopped a coronavirus outbreak before it spread by testing students' poop

University of Arizona catches asymptomatic coronavirus cases through wastewater testing

WBE accuracy as an early-warning diagnostic for new cases of COVID-19

			Clinical Results	
			Positive	Negative
Wastewater Results	↕	Positive	91	20
		Negative	23	185

Sensitivity (79.8%)

Specificity (90.2%)

Positive predictive value (82.0%)

Negative predictive value (88.9%)

% ASYMPTOMATIC CASES

- Testing all residents in a dorm allowed for documentation of symptomatic and asymptomatic infections
- **Analysis of data showed that 80% of the cases were asymptomatic**

EFFECT ON HERD IMMUNITY

- Nationwide if 80% of all cases were asymptomatic, then true number of infections much greater than those reported
- 31 million infections recorded, but this would only be 20% of true number
- Real number = 155 million
- Current \approx 130m people vaccinated so total # people with immunity = 285 million
- Are we currently experiencing herd immunity?

WBE TO ESTIMATE DISEASE PREVALENCE IN LARGER COMMUNITIES

- CDC Best Estimate = Reported # of cases x 4.3
- Interestingly, the CDC Best Estimate predicts **72% asymptomatic cases**
- Now use the Curtis Equation

CURTIS EQUATION

- CDC Best Estimate # Infections =

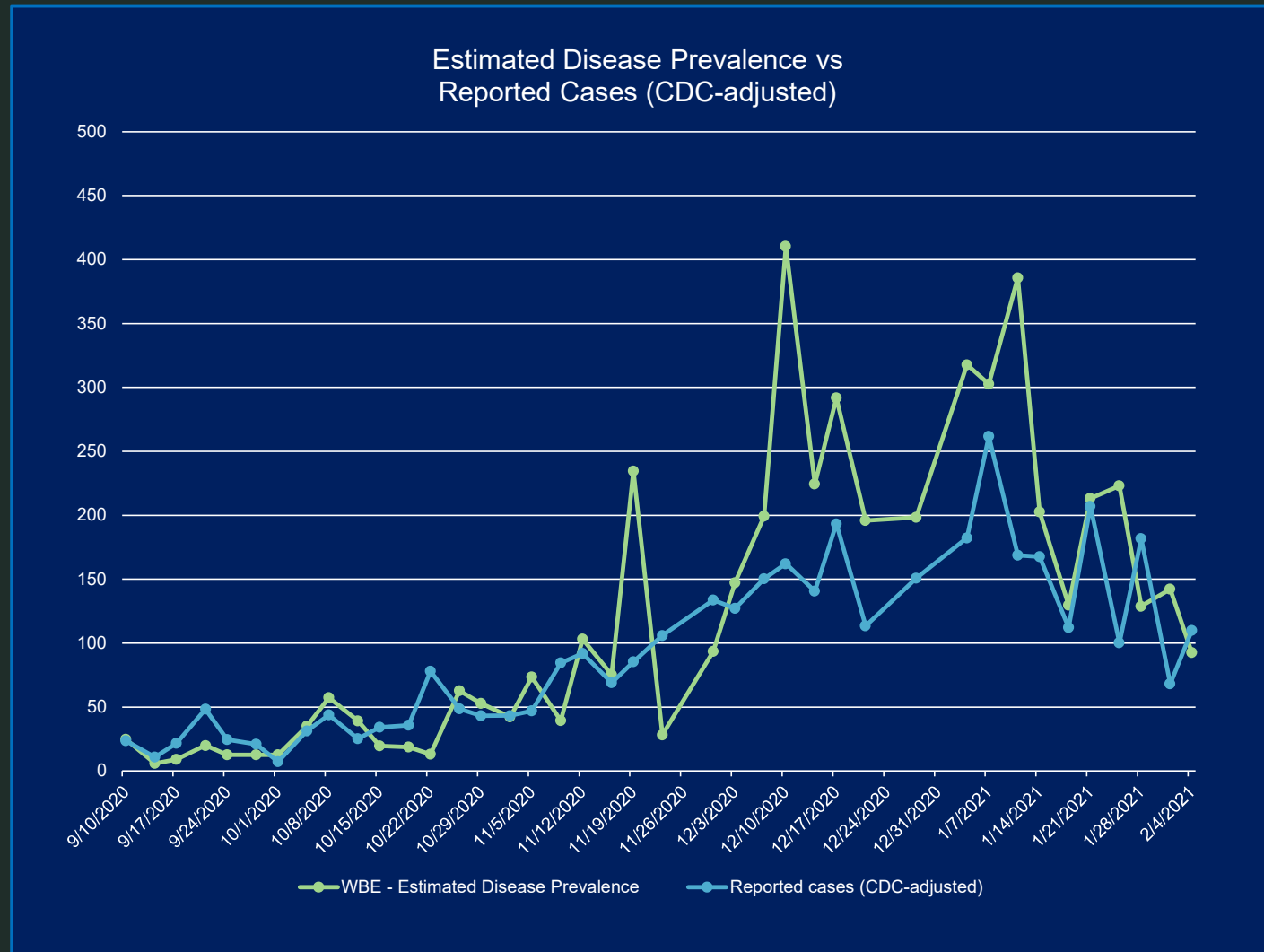
$$\frac{\text{Sewage concentration} \times \text{Wastewater flow rate}}{\text{Amount of feces/person} \times \text{Fecal shedding rate}}$$

- Calculate Fecal shedding rate
- Repeat 20-30 times to obtain mean shedding rate

DEMOGRAPHIC EFFECTS ON SHEDDING RATE

- Illness severity and viral load/shedding generally increase with age
- **Must determine the mean shedding rate for any given community (utility service area)**
- Can then use the Curtis Equation for future wastewater concentrations to estimate disease prevalence (EDP)

Wastewater-Based Epidemiology (WBE) for Estimation of Disease Prevalence



OTHER USES OF WBE FOR COVID TESTING

- Hot zone monitoring of high-risk populations
 - Schools
 - Nursing homes
 - Retirement homes
 - Prisons
 - Military bases
 - Work crews (via Port-a-Potties)
- Shotgun viral metagenomics to detect new/emerging viral pathogens

MONITORING FOR CORONAVIRUS VARIANTS

- Out of control in many parts of the world such as India, Europe, & U.S.
- VOCs
 - more transmissible
 - affecting younger populations
 - more deadly
- **Variants of Concern (VOC):**
 - Alpha (British B.1.1.7)
 - Beta (South African B.1.351)
 - Gamma (Brazilian P.1)
 - Delta (Indian B.1.617)
 - Epsilon (California B.1.427/B.1.429) = Variant of Interest
- Sequencing may be required to distinguish which specific variant(s) is present.

Agua Nueva Wastewater-Based Epidemiology: Variant Screening

Sample ID	ANWRF	ANWRF	ANWRF	ANWRF
Date	2/11/2021	2/24/2021	3/3/2021	3/11/2021
N1 test result (GC/L)	7.63E+05	5.60E+05	5.33E+05	5.42E+05
N2 test result (GC/L)	9.02E+05	2.83E+05	4.49E+05	4.01E+05
Presumptive variant	B.1.1.7	B.1.1.7	B.1.1.7	B.1.1.7
Variant analysis targets	N501Y/DEL69-70	N501Y/DEL69-70	N501Y/DEL69-70	N501Y/E484K
Variant test results	4.10E+02/Detect	1.3E+03/Detect	1.70E+03/Detect	2.29E+03/Non-detect
Kit used	GT-MOLECULAR	GT-MOLECULAR	GT-MOLECULAR	BIO-RAD

Sample ID	ANWRF	ANWRF	ANWRF	ANWRF
Date	3/17/2021	3/17/2021	3/22/2021	3/22/2021
N1 test result (GC/L)	1.11E+06	1.11E+06	1.03E+06	1.03E+06
N2 test result (GC/L)	3.39E+05	3.39E+05	1.23E+06	1.23E+06
Presumptive variant	N/A	P.1/B.1.351	B.1.1.7/P.1/B.1.351	B.1.1.7/P.1/B.1.351
Variant analysis targets	N501Y/DEL69-70	N501Y/E484K	N501Y/E484K	N501Y/E484K
Variant test results	Non-detect/Non-detect	Non-detect/ 6.79E03	1.29E+03/Detect	Detect/1.70E+03
Kit used	GT-MOLECULAR	BIO-RAD	BIO-RAD	BIO-RAD

Note: When multiple variants are indicated and/or uncertainty exists, sequencing is needed to clarify.



WASTEWATER-BASED EPIDEMIOLOGY

POOP NEVER LIES!

University of Arizona