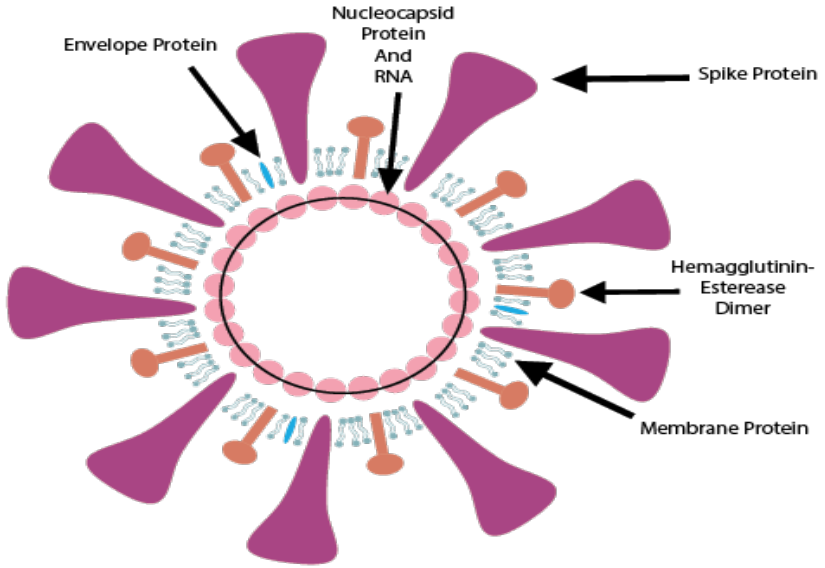
Wastewater-based epidemiology approach for COVID-19 virus detection



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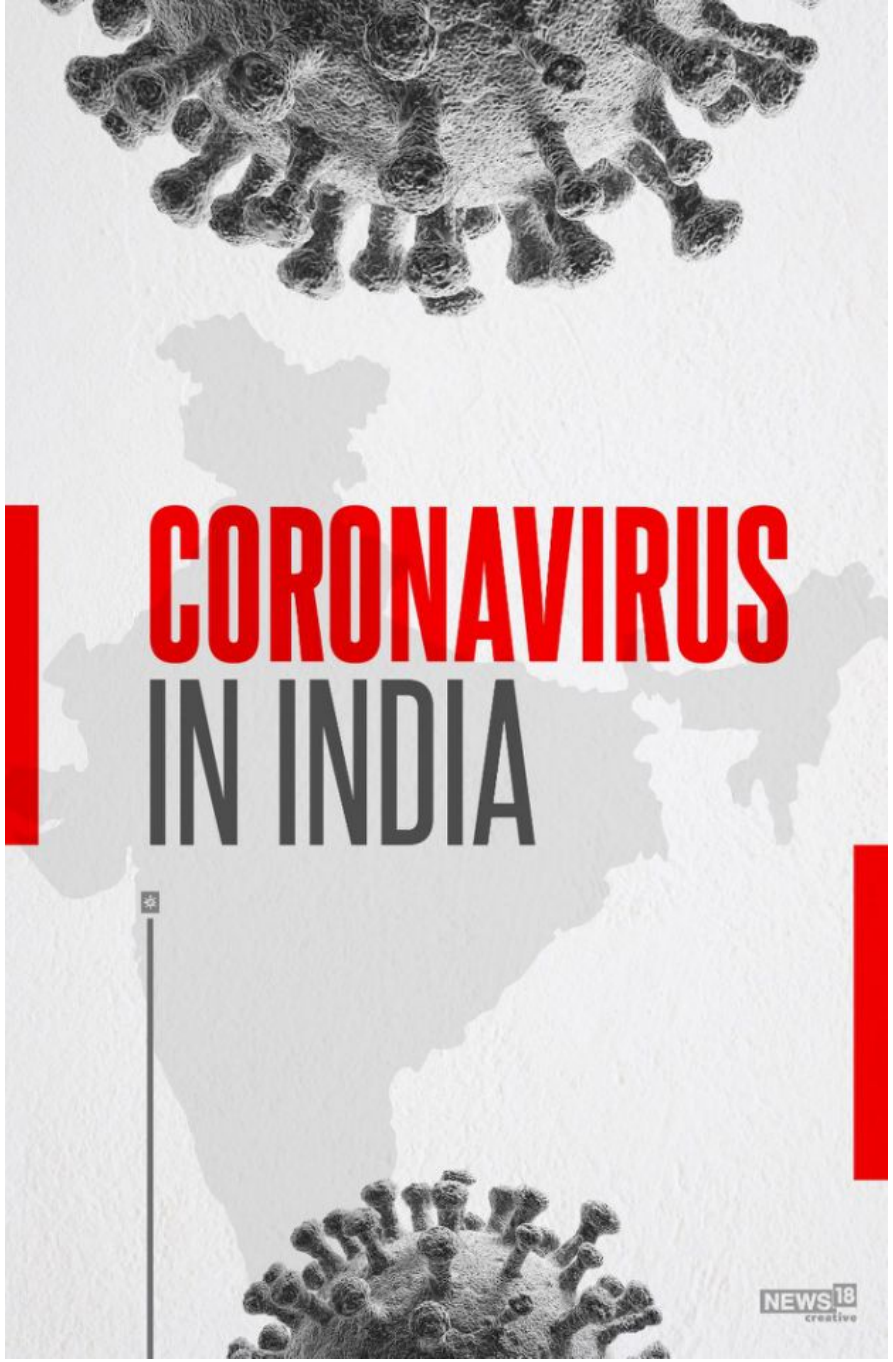
Outline of Presentation

- Covid-19 Timeline in India
- History of Coronaviruses
- COVID-19 in Wastewater
- Wastewater-based epidemiology (WBE) and its significance
- WBE approach and its applications
- WBE: Potential method for disease surveillance
- SARS Co-V-2 in wastewater: Detection and elimination
- COVID-19: Post pandemic menace
- Conclusions


CORONAVIRUS
IN INDIA
NEWS18
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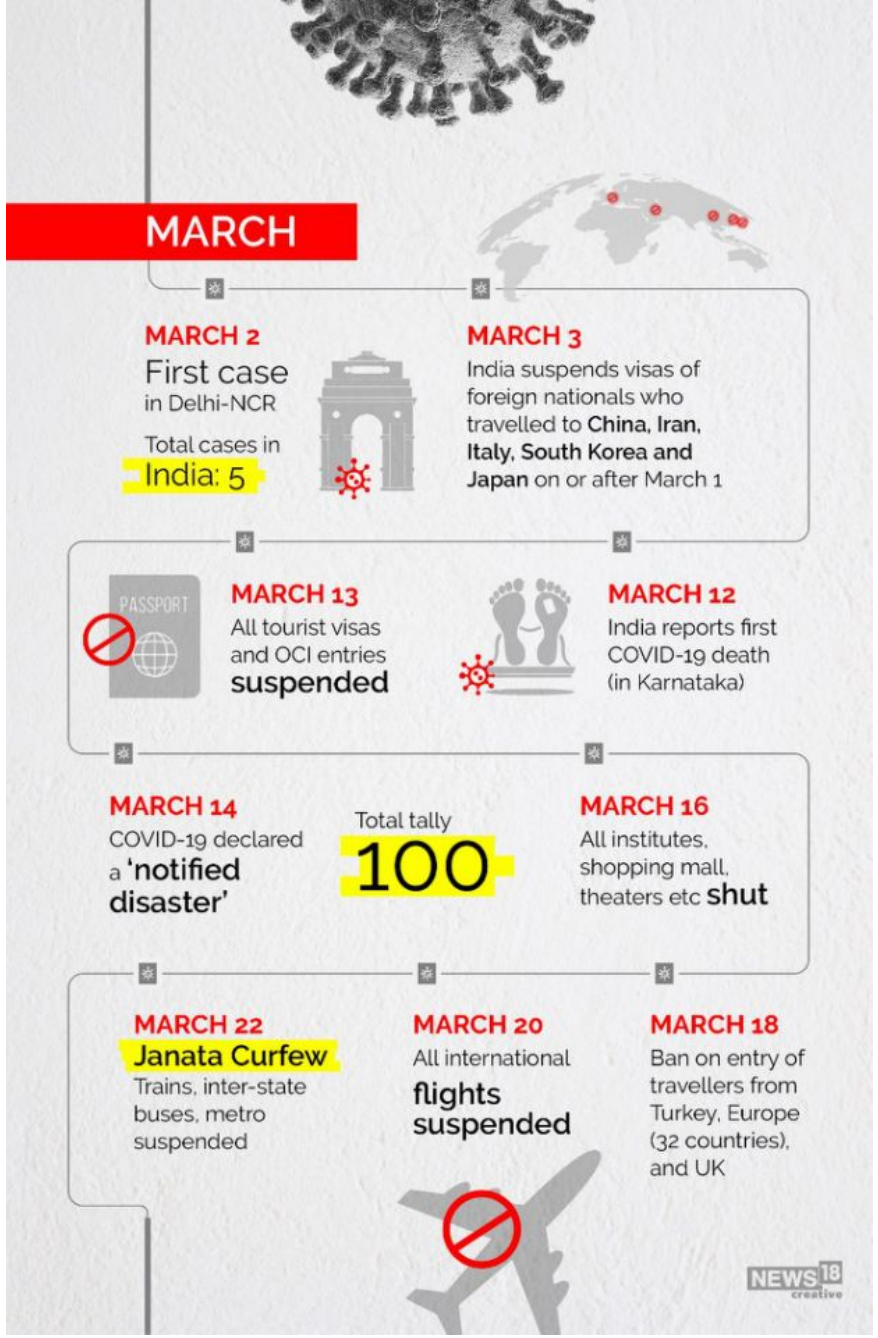
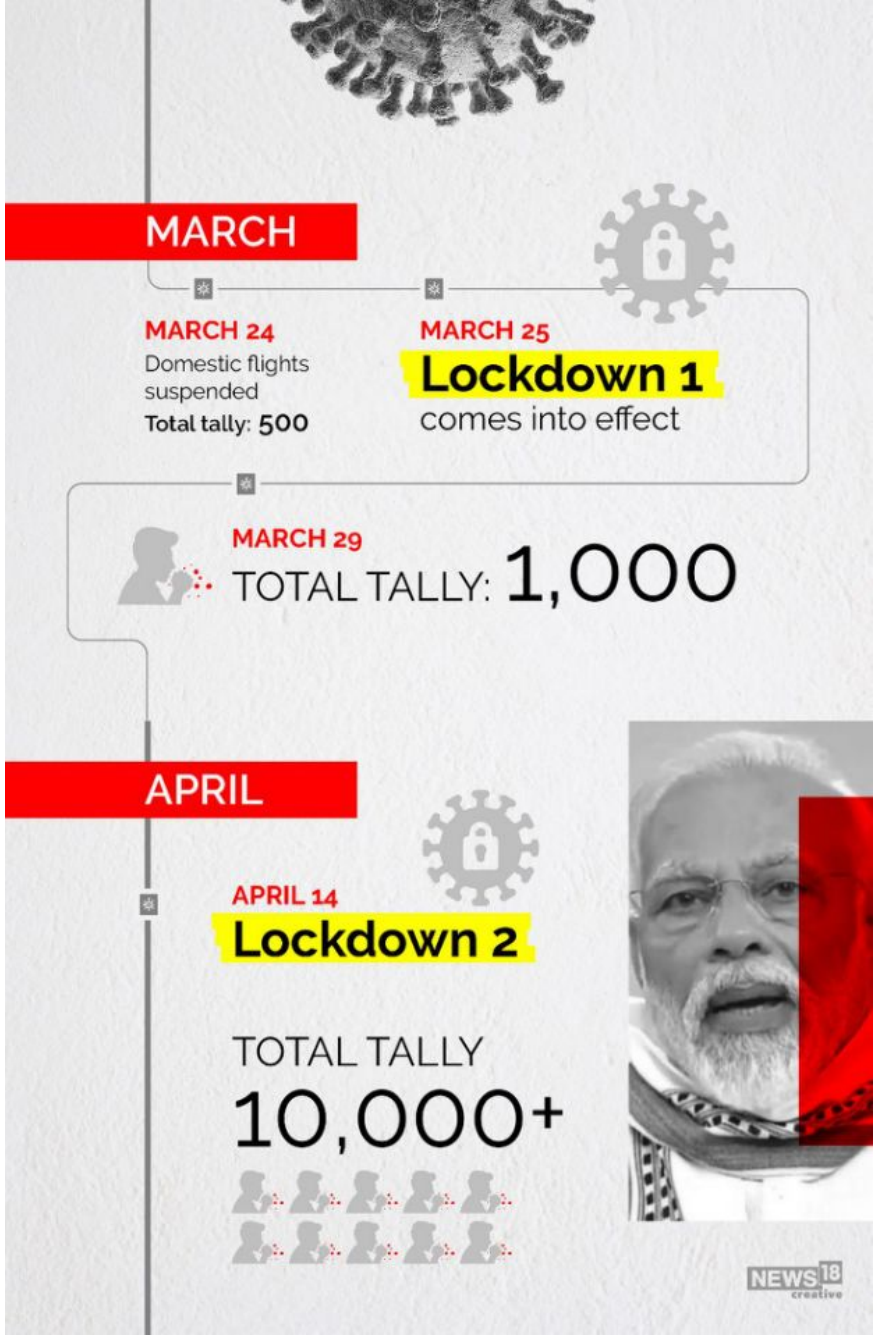
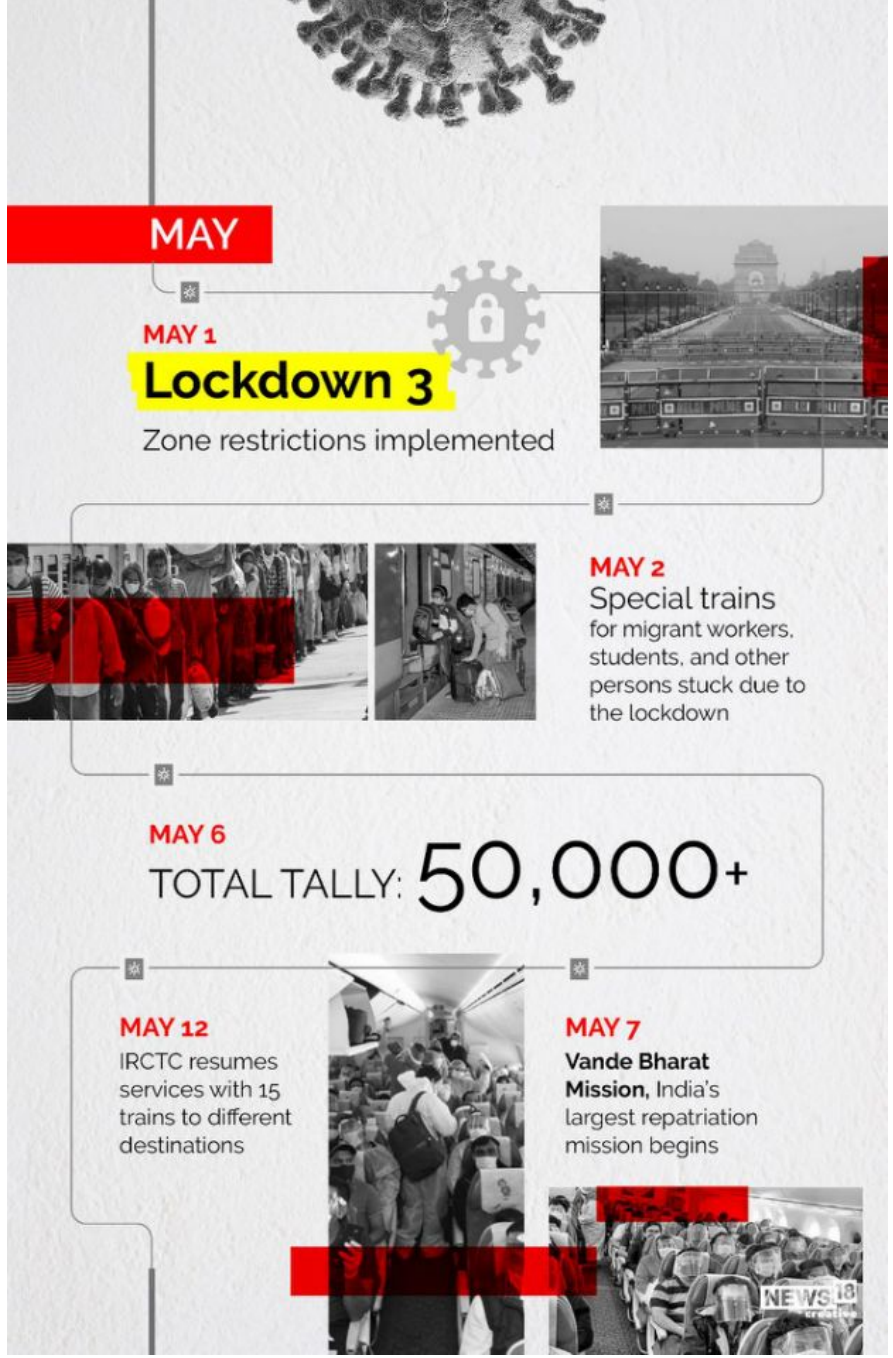
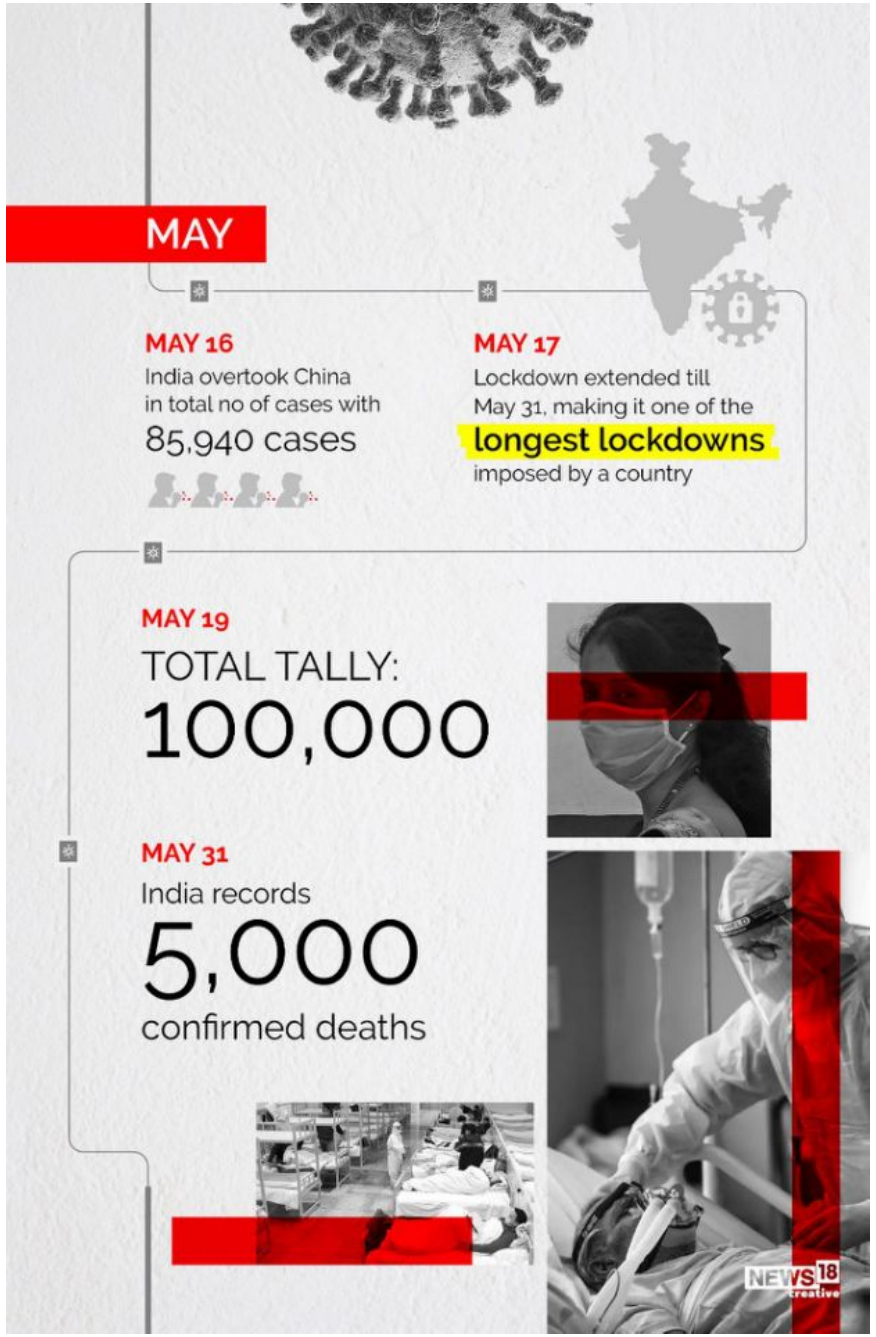
JANUARY 30
First confirmed
case reported (in Kerala)
Coronavirus :
FEBRUARY 3
Third case in Kerala
Govt declares state
emergency
NEWS18
creative

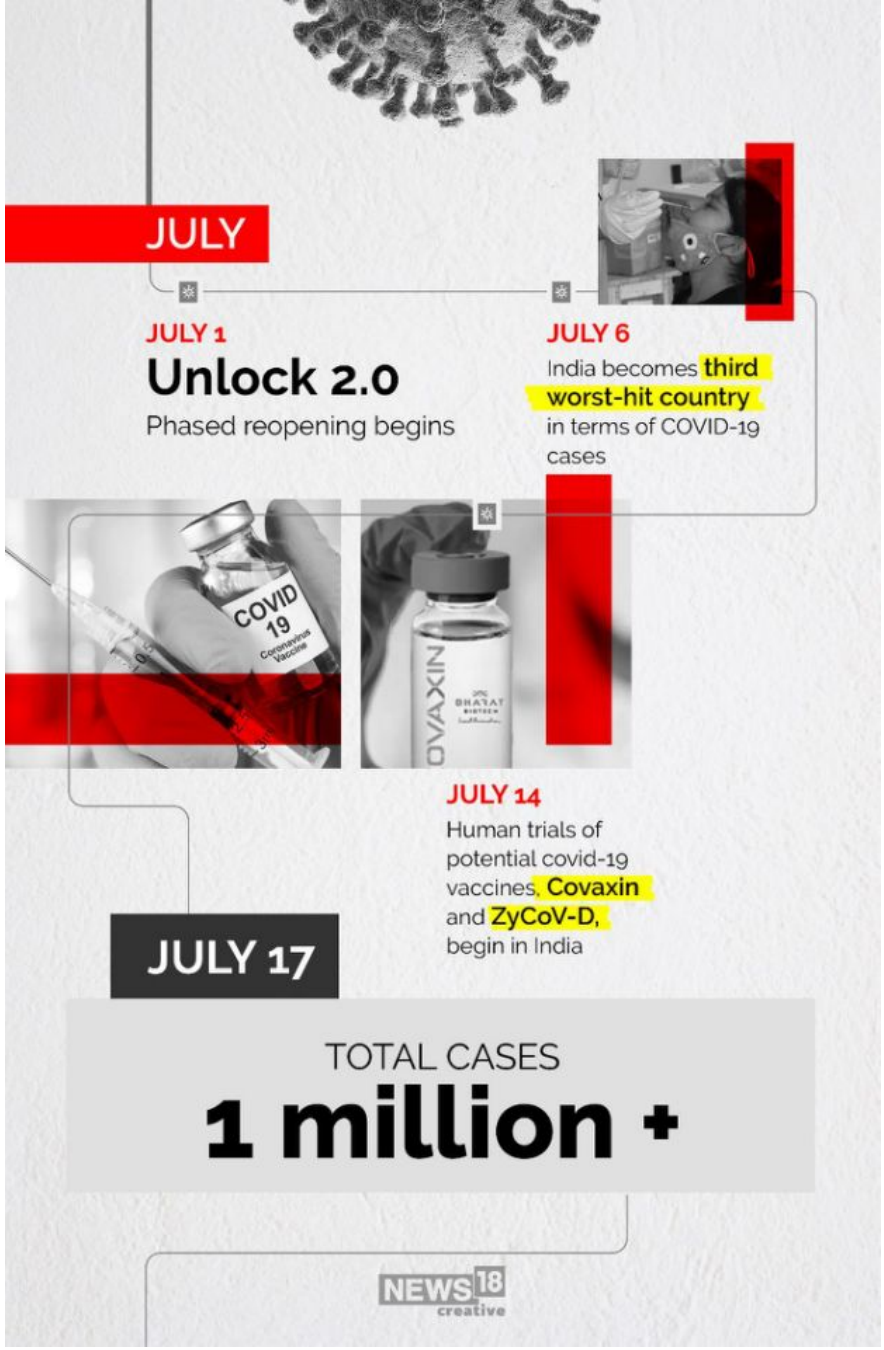

MARCH
MARCH 2
First case
in Delhi-NCR
Total cases in
India: 5
MARCH 3
India suspends visas of foreign nationals who travelled to China, Iran, Italy, South Korea and Japan on or after March 1
PASSPORT
MARCH 13
All tourist visas and OCI entries suspended
MARCH 12
India reports first COVID-19 death (in Karnataka)
MARCH 14
COVID-19 declared a 'notified disaster'
Total tally
100
MARCH 16
All institutes, shopping mall, theaters etc shut
MARCH 22
Janata Curfew
Trains, inter-state buses, metro suspended
MARCH 20
All international flights suspended
MARCH 18
Ban on entry of travellers from Turkey, Europe (32 countries), and UK
NEWS18 creative

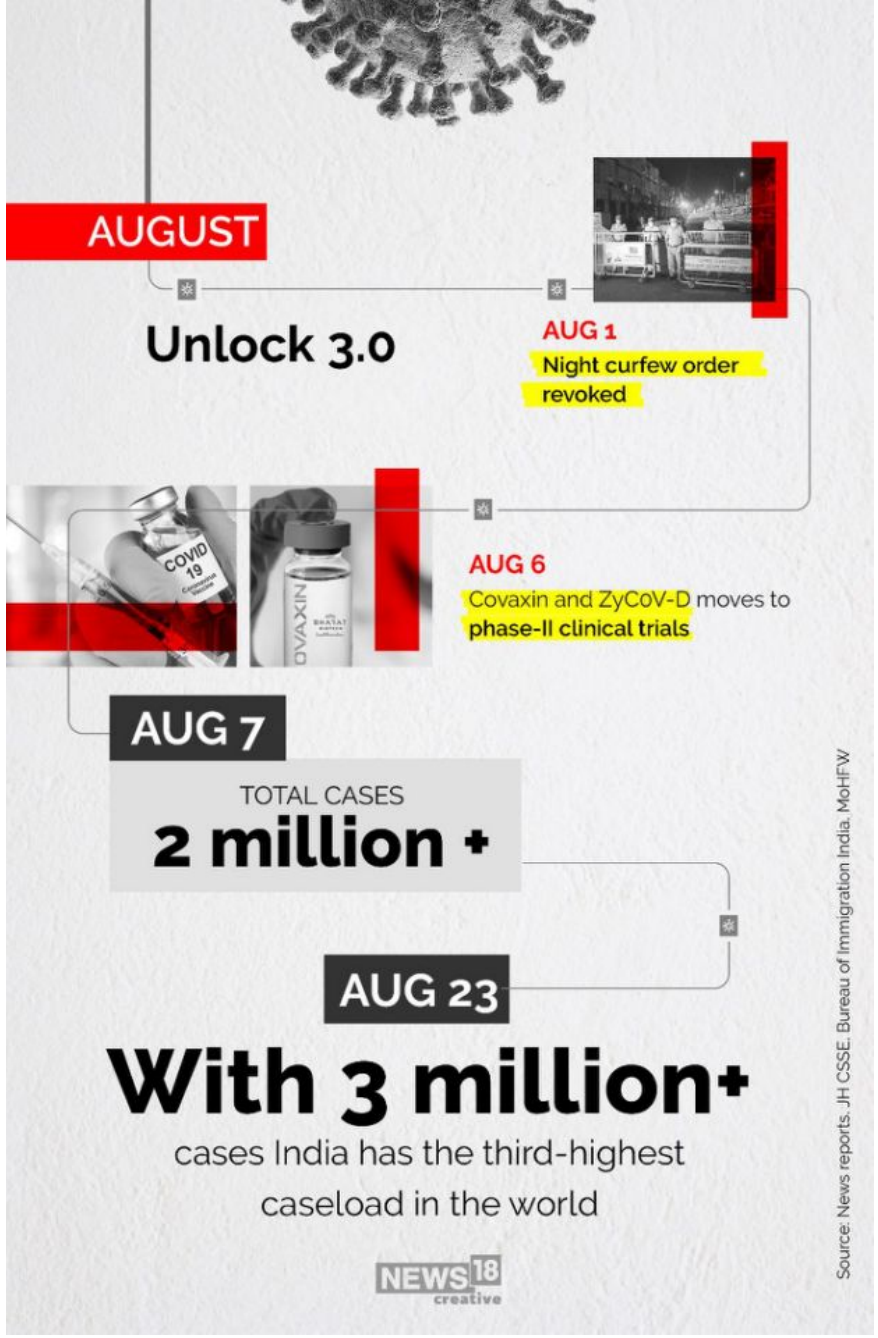

MARCH
MARCH 24
Domestic flights suspended
Total tally: 500
MARCH 25
Lockdown 1
comes into effect
MARCH 29
TOTAL TALLY: 1,000
APRIL
APRIL 14
Lockdown 2
TOTAL TALLY
10,000+
NEWS18 creative


MAY
MAY 1
Lockdown 3
Zone restrictions implemented
MAY 2
Special trains
for migrant workers, students, and other persons stuck due to the lockdown
MAY 6
TOTAL TALLY: 50,000+
MAY 12
IRCTC resumes services with 15 trains to different destinations
MAY 7
Vande Bharat Mission, India's largest repatriation mission begins
NEWS18


MAY
MAY 16
India overtook China in total no of cases with
85,940 cases
MAY 17
Lockdown extended till May 31, making it one of the
longest lockdowns
imposed by a country
MAY 19
TOTAL TALLY:
100,000
MAY 31
India records
5,000
confirmed deaths
NEWS18 creative


JUNE
JUNE 1
IRCTC starts 200 special trains
JUNE 8
Unlock 1.0
Phased reopening begins
JUNE 25
CBSE cancels all remaining board exams
JUNE 17
India registers 2,003 deaths
(Highest-ever as Maha and Delhi add 'backlog' fatalities to their data)
JUNE 11
India becomes the fourth worst-affected country
JUNE 27
TOTAL TALLY:
500,000+
NEWS18 creative


JULY
JULY 1
Unlock 2.0
Phased reopening begins
JULY 6
India becomes third worst-hit country in terms of COVID-19 cases
COVID 19
Coronavirus Vaccine
COVAXIN
JULY 14
Human trials of potential covid-19 vaccines, Covaxin and ZyCoV-D, begin in India
JULY 17
TOTAL CASES
1 million +
NEWS18 creative


AUGUST
Unlock 3.0
AUG 1
Night curfew order revoked
COVID 19
COVAXIN
AUG 6
Covaxin and ZyCoV-D moves to phase-II clinical trials
AUG 7
TOTAL CASES
2 million +
AUG 23
With 3 million+
cases India has the third-highest caseload in the world
NEWS18 creative
Source: News reports, JH CSSE, Bureau of Immigration India, MoHFW

Countries	Confirmed	Deaths	Recoveries
India	3,936,747	68,472	3,037,151
United States	6,156,658	186,794	2,283,454
Brazil	4,092,832	125,521	3,278,918
Russia	1,015,105	17,649	832,747
Peru	670,145	29,405	489,886
Colombia	650,062	20,888	498,221

Updated 9/05/2020 - from multiples sources that refresh at various times

History of Coronaviruses

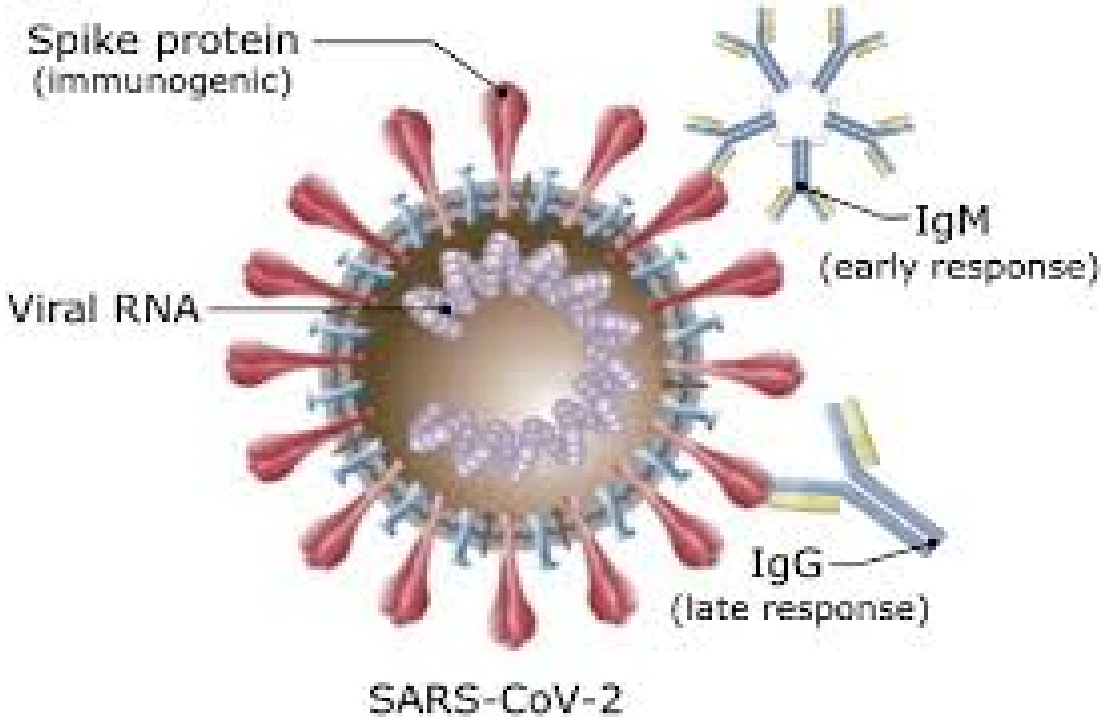
- Coronaviruses: Large family of viruses having ability to cause illnesses with wide range of severity.
- 1st known server illness by a coronavirus: Emerged in 2003 with Severe Acute Respiratory Syndrome (SARS) epidemic in China.
- 2nd outbreak of severe illness by coronavirus: 2012 in Saudi Arabia with Middle East Respiratory Syndrome (MERS).
- Novel SARS-CoV-2 coronavirus: Emerged in December 2019 Wuhan, China also known as COVID-19

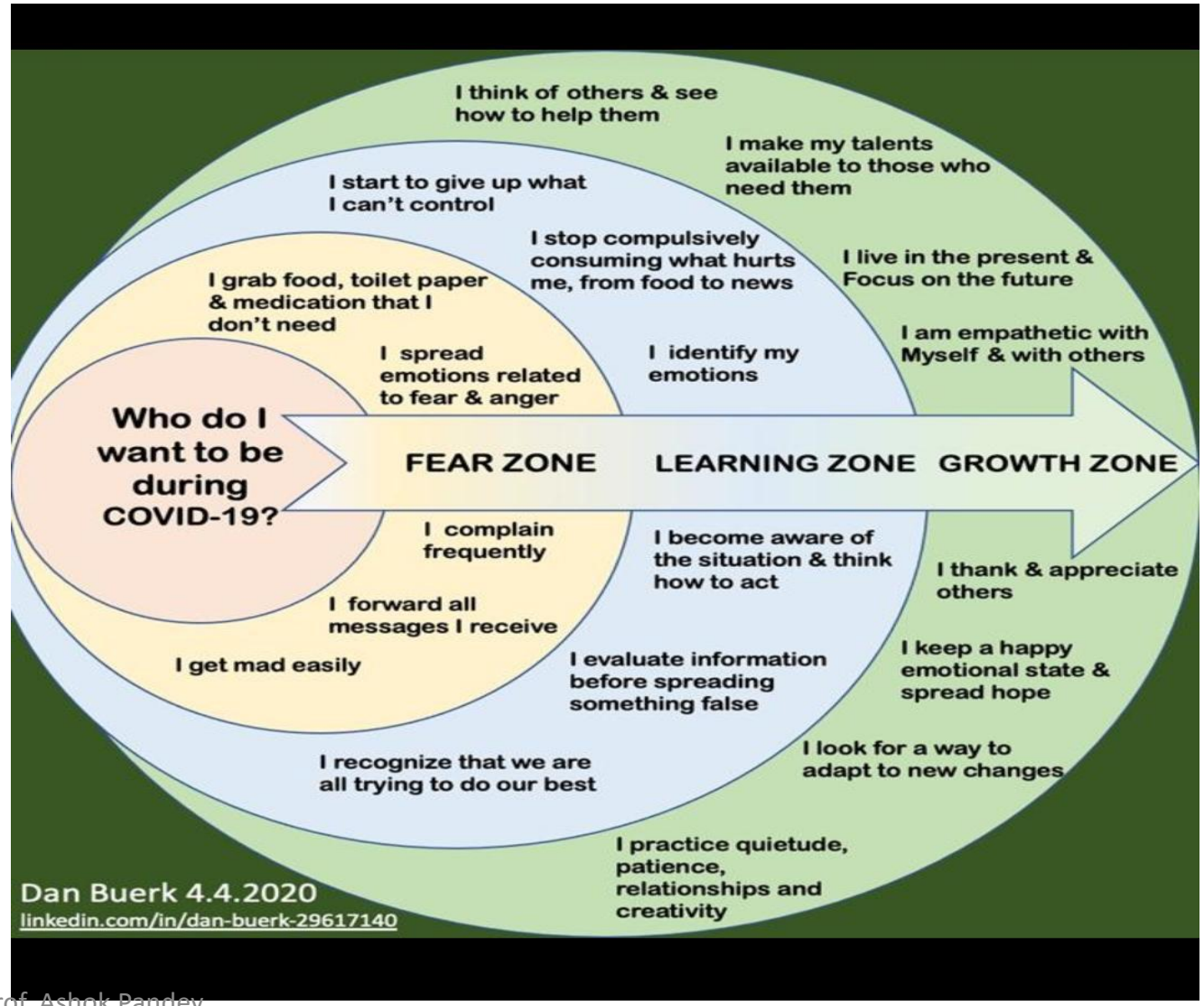
COVID-19

- COVID-19 : Disease caused by a new strain of coronavirus.
- COVID-19: 'CO' stands for corona, 'VI' for virus, 'D' for disease and '19' for 2019
- Formerly, this disease was referred to as '2019 novel coronavirus' or '2019-nCoV'
- Coronavirus disease (COVID-19) pandemic: Caused by infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
- Active replication of infectious SARS-CoV-2 particles in enterocytes of human intestine occurs due to expression of ACE2 receptor and causes shedding of virus in the faeces

COVID-19

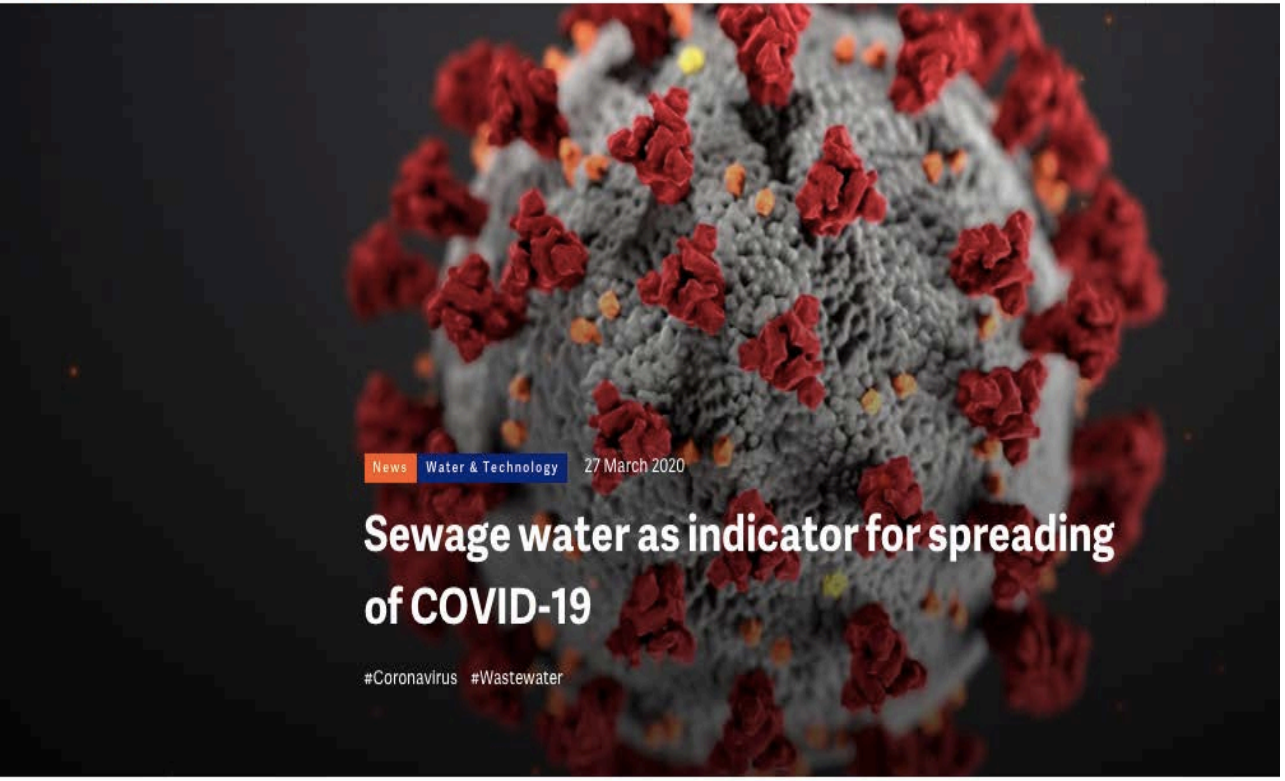
- Clinically reported symptoms in COVID-19 patients mainly
  - Cough
  - Difficulty in breathing
  - Fever
  - Diarrhea





COVID 19 in Wastewater

Netherlands News Dutch Water Sector



27th March 2020, Netherlands

Microbiologists at research institute KWR conducted a series of RNA-analyses at municipal waste water treatment plants (WWTP) in the Netherlands. The analyses showed the presence of RNA gene fragments of the COVID-19 virus in incoming sewage water.

According to KWR the screening of the COVID-19 virus at municipal waste water plants can be used to signal new outbreaks in advance and play an important role to follow the evolution of the pandemic.

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Additional research

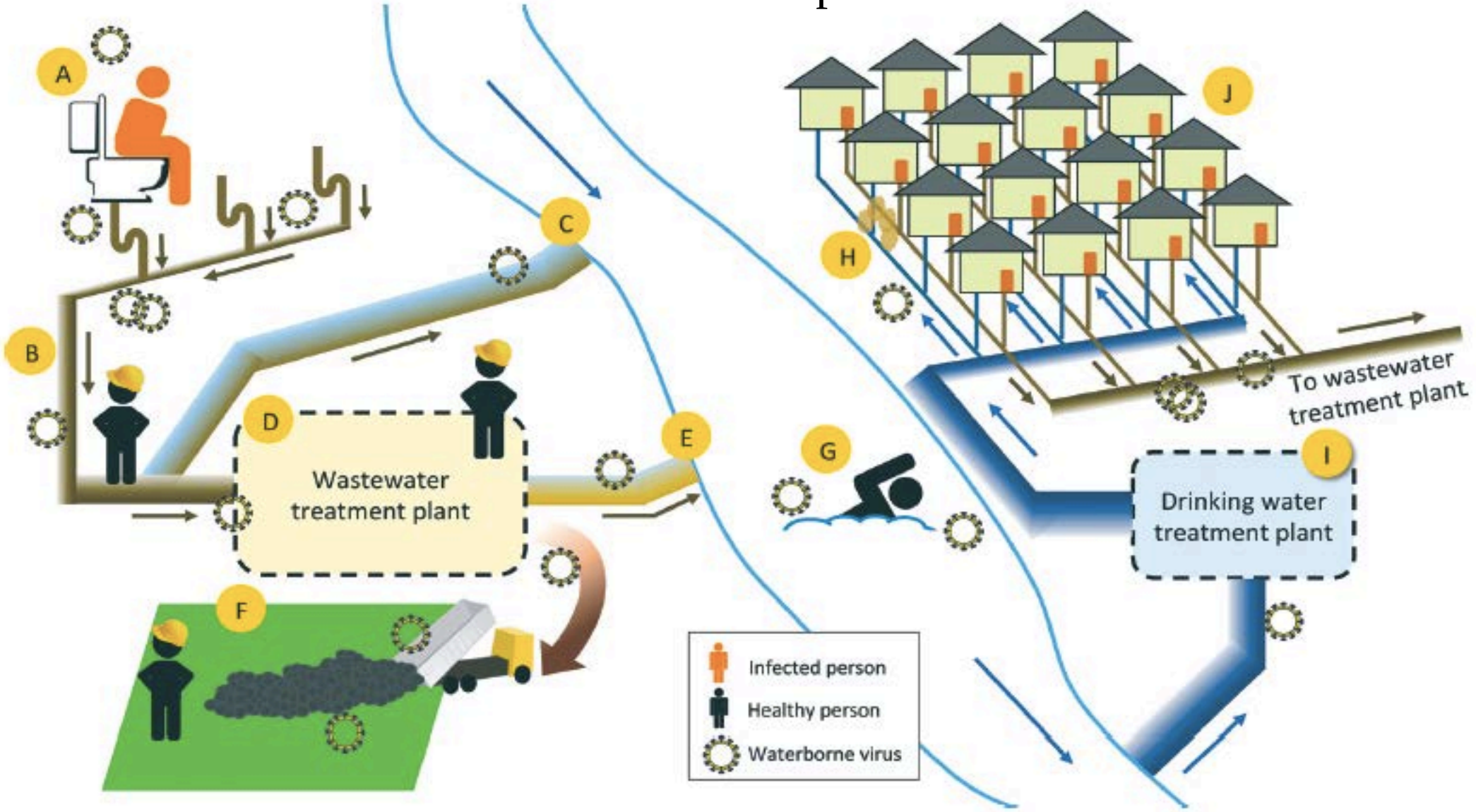
RNA-analysis is a method to measure the presence of viruses through capturing virus particles and detect specific gene fragments. The method does not discriminate between inactive and infectious particles. The KWR microbiologists say they have not yet been able to quantify the presence of these fragments. Their first findings indicate that the concentration of the virus at the WWTP is low.

Currently researchers are examining all samples multiple times and are looking at the reproducibility of the results. Furthermore, they double check and focus on fragments of multiple genes, to strengthen their results about the presence of the virus.

Gene fragments of COVID-19 detected at Dutch WWTP.

Source: https://www.dutchwatersector.com/news/sewage-water-as-indicator-for-spreading-of-covid-19

The fate of infective viruses in the urban water cycle and locations of potential human exposure



(**Source:** Wigginton et al., 2015; DOI: 10.1039/c5ew00125k)

Wastewater-based epidemiology (WBE)

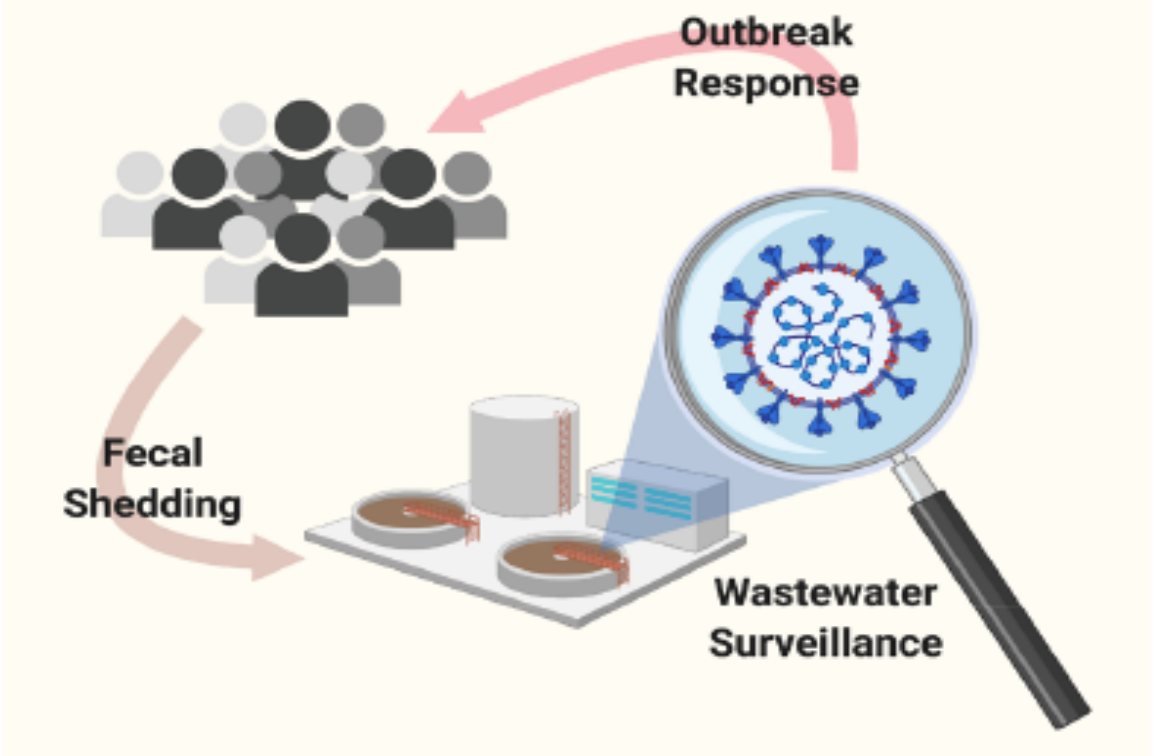


Figure 1. In wastewater-based epidemiology (WBE), the prevalence of SARS-CoV-2 infections in a community could be estimated by enumerating the virus RNA in that community's sewage and performing mass balances on virus shedding using population and sewage flow rate data. Such information can then inform public health responses to the outbreak.

- Wastewater-based epidemiology (WBE) for detection of COVID-19 has gained global attention
- Many scientific reports indicate that the concentration of SARS-CoV-2 in wastewater is proportional to the number of COVID-19 patients in a sewer shed.

Source: Bivins et al. (2020). Environ. Sci. Technol. 2020, 54, 7754−7757

WBE: Significance

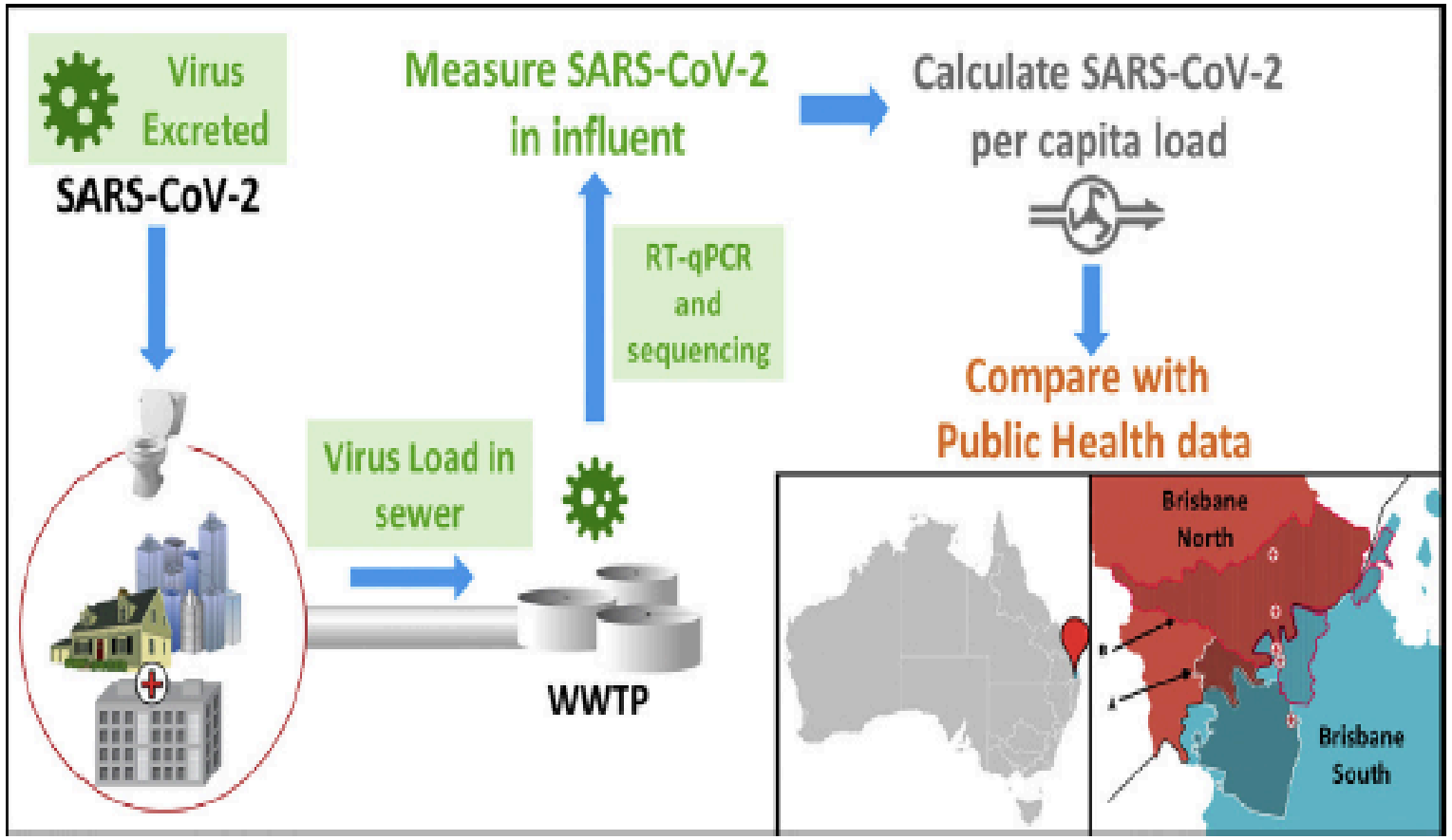
- WBE has been used in forensic science studies and surveillance of polio virus cases in India for a long period.
- The conventional epidemiology depends on systematic diagnosis of samples and clinical symptoms.
- It is hard to detect the highly infectious disease in a large population in a timely manner.
- Published literature: Demonstrate asymptomatic individuals along with symptomatic patients, discharge virus/viral material which ultimately reaches wastewater treatment plants (STP).
- It can be shed in faeces for several days, even after the patient stops exhibiting respiratory symptoms.
- WBE: promising approach to study/understand disease outbreak status in wastewater.

Wastewater-based epidemiology (WBE)

HIGHLIGHTS

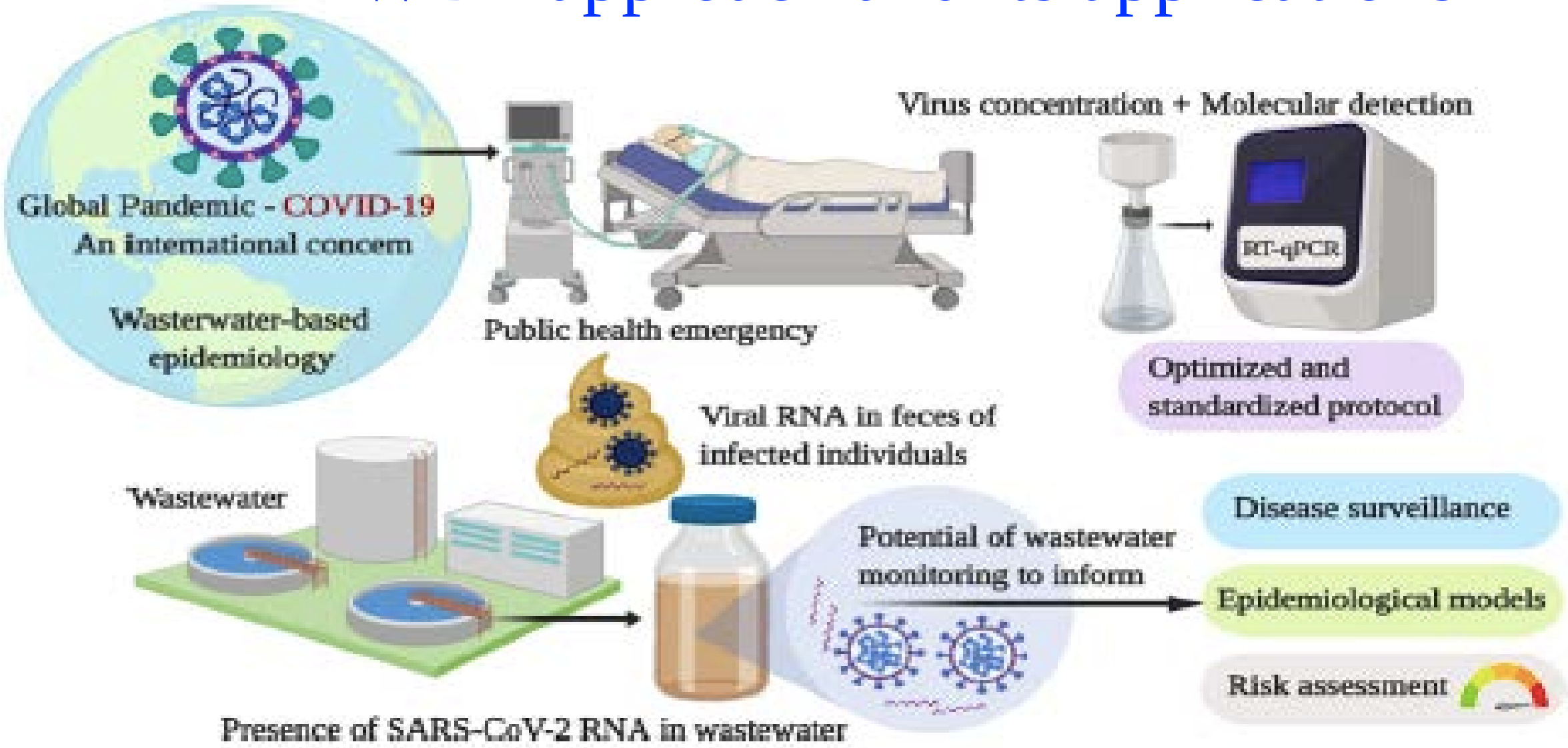
- First study that reports the detection of SARS-CoV-2 in wastewater in Australia.
- The presence of SARS-CoV-2 was confirmed by sequencing.
- A median range of 171 to 1090 infected persons was identified in the catchment.
- Further methodological and molecular assay validation will be required.

GRAPHICAL ABSTRACT



Source: Ahmed et al., (2020), Science of the Total Environment 728 (2020) 138764

WBE approach and its applications



Source: Kitajima et al., 2020

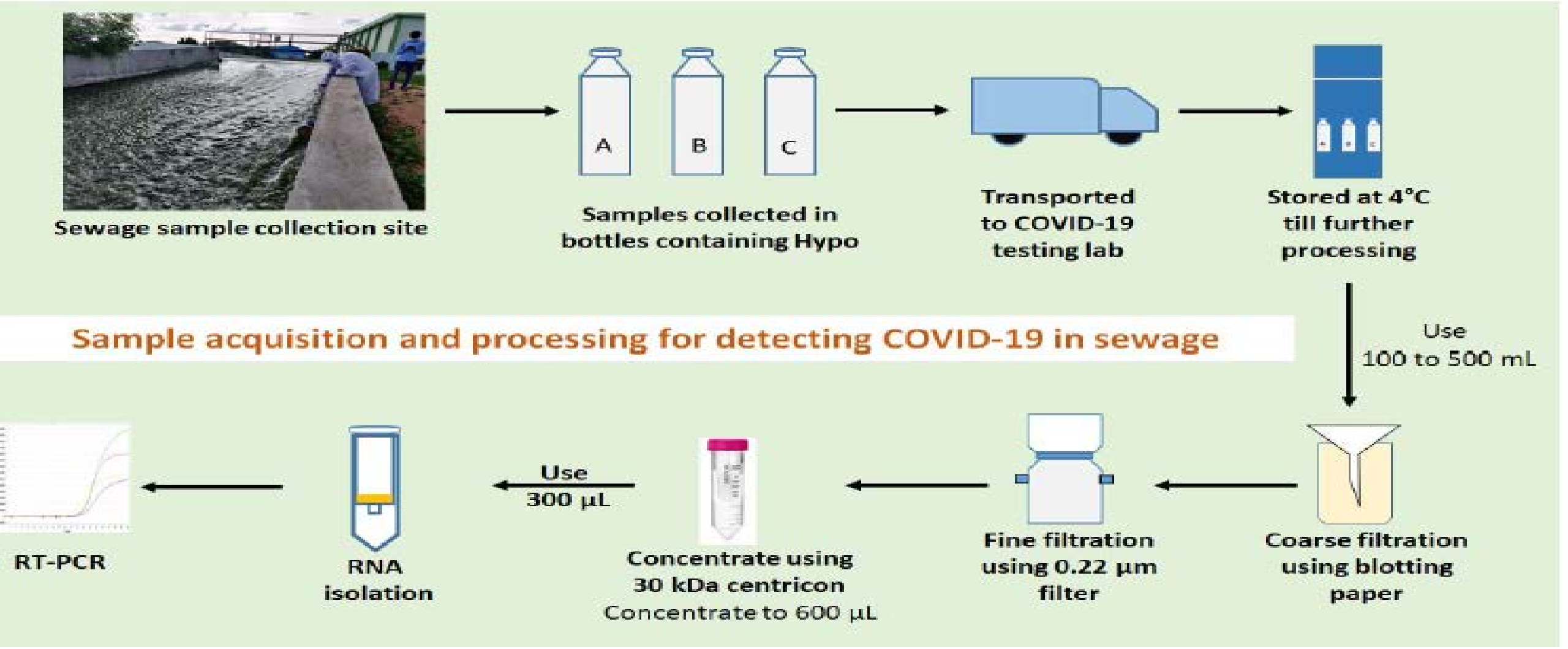
SARS Co-V-2 in wastewater

Table 2
Details of reported molecular detection of SARS-CoV-2 in wastewater.

Sampling location		Water type	Virus detection methods			Detection results		Reference
Country	State/city		Virus concentration method	qPCR assay[a]	Sequence confirmation	Positive rate	Maximum concentration (copies/L)	
Australia	Brisbane, Queensland	Untreated wastewater	Electronegative membrane-direct RNA extraction; ultrafiltration	N_Sarbeco NIID_2019-nCOV	Direct sequence of qPCR products (Sanger + MiSeq)	2/9 (22%)	1.2×10^2	(Ahmed et al., 2020)
The Netherlands	Amsterdam, The Hague, Utrecht, Apeldoorn, Amersfoort, Schiphol, Tilburg	Untreated wastewater	Ultrafiltration	CDC N1, N2, N3 E_Sarbeco	Not done	14/24 (58%)	Not available	(Medema et al., 2020)
USA	Massachusetts	Untreated wastewater	PEG precipitation	CDC N1, N2, N3	Direct sequence of qPCR products (Sanger)	10/14 (71%)	$>2 \times 10^5$	(F. Wu et al., 2020b)
France	Paris	Untreated wastewater	Ultracentrifugation	E_Sarbeco	Not done	23/23 (100%)	$>10^{6.5}$	(Wurtzer et al., 2020)
		Treated wastewater	Ultracentrifugation	E_Sarbeco	Not done	6/8 (75%)	$\sim 10^5$	
USA	Bozeman, Montana	Untreated wastewater	Ultrafiltration	CDC N1, N2	*Re*-amplification by regular PCR followed by Sanger sequencing	7/7 (100%)	$>3 \times 10^4$	(Nemudryi et al., 2020)

(**Source:** Kitajima et al., 2020)

WBE: Potential method for disease surveillance



Sample acquisition and processing for detecting COVID-19 in sewage

Figure 1: Schematic of sample collection and processing: The sewage samples are collected from STPs in bottles containing Hypo (0.1-4%) followed by shipping to the COVID-19 testing laboratory and stored at 4 °C until further processing. The samples are initially filtered using blotting paper followed by 0.22 µm filter. These filtered samples are further concentrated using 30 kDa centricon filters. The concentrates are used for RNA isolation, the isolated RNA is subjected to RT-PCR using COVID-19 specific primers and probes.

Source: https://www.medrxiv.org/content/10.1101/2020.08.18.20177428v1.article-metrics

WBE: Potential method for disease surveillance

- Above study provided a concrete evidence for application of WBE as a potential method for disease surveillance.
- Study was performed in STPs of Hyderabad for presence of SARS-CoV-2 genome traces in wastewater.
- Presence of SARS-CoV-2 genetic material in allstudied STPs was detected and extent of spread by estimating the number of infected people and possible number of active cases was calculated.
- The results may be a resource for healthcare and associated departments to vigilantly allocate necessary resources to manage existing cases as well as to carefully contain disease spread.
- Hence, sewage-based surveillance: Holistic approach to manage the pandemic and also to monitor for future outbreaks.

Source: https://www.medrxiv.org/content/10.1101/2020.08.18.20177428v1.article-metrics

Paper Based Detection to Trace COVID-19

The direct method for detection of SARS-Co-V-2 is Polymerase chain reaction (PCR) assay (A nucleic acid-based method)

PCR assay salient features:

- Highly sensitive and specific
- Skilled manpower required
- Long period of data processing and analysis (Approx 5 hrs)
- Not conducive to real time

Hence it is necessary to develop robust, speedy and transposable analytical approach/tool for accurate and swift tracing of low concentration of SARS-Cov-2 in wastewater samples through WBE approach

PAPER BASED ANALYTICAL DEVICES

Testing Process: Simple folding of a paper-based device in different ways in different steps

Paper based analytical devises:

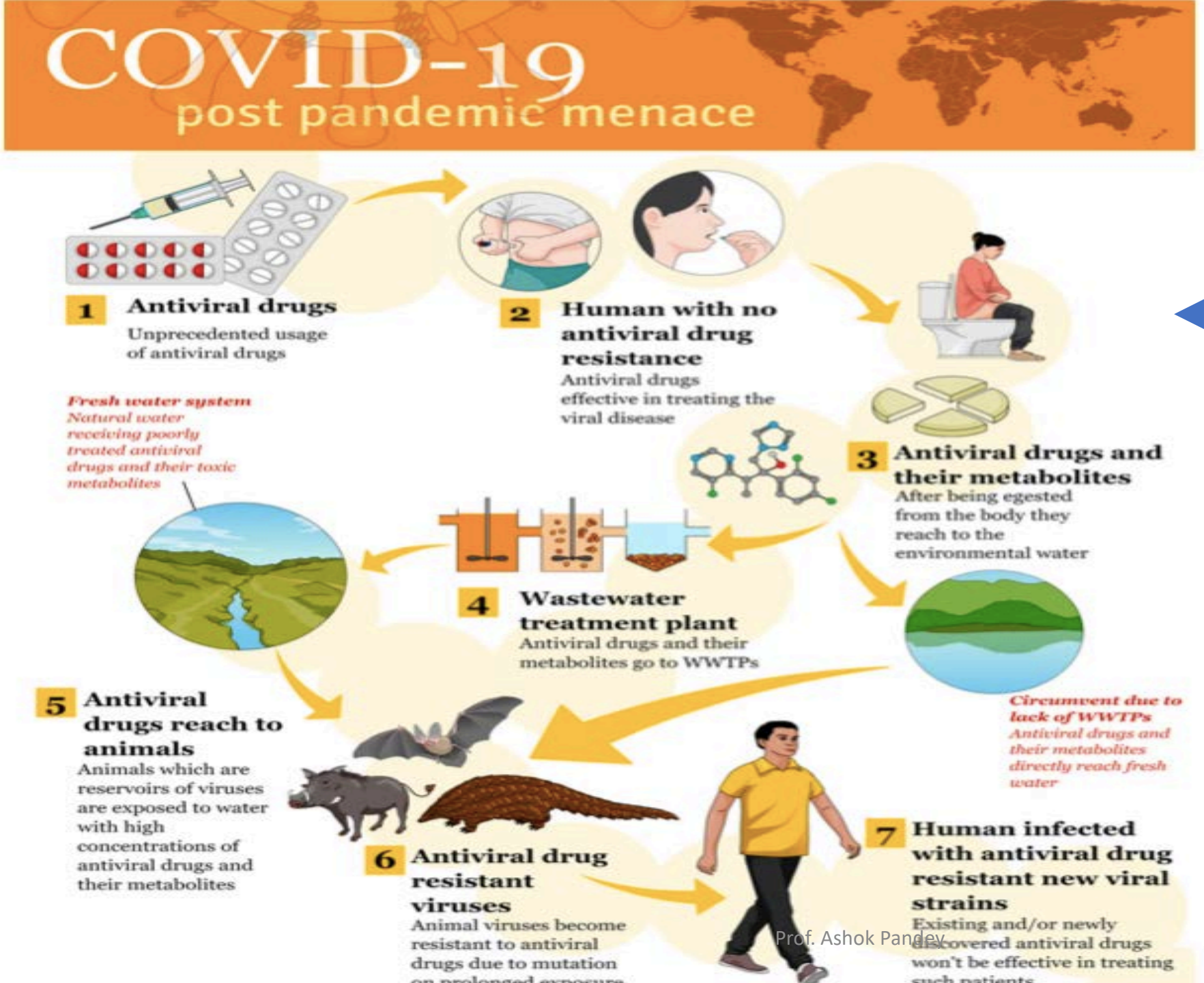
- No pump or power supply required.
- Overcomes limitations of PCR
- Avoid multiple processes
- Enables multiplexed, sensitive assays, with high quality and fast precision

(**Source:** Mao et al., 2020, Environ. Sci. Technol. 2020, 54, 3733−3735

Table 1. Examples of Paper-Based Devices for Infectious Diseases and Pathogens Determination

infectious diseases/pathogens	characteristics of paper-based devices	detection method
malaria	paper device combined vertical flow sample-processing steps	visual UV/lateral flow device
rotavirus A	integrated nucleic acid test on a single paper device, including extraction, amplification, and on-site detection	naked eye
Zika virus	wax-printed paper devices utilizing isothermal amplification	smartphone
human papillomavirus	paper device in a foldable system allowing for fully integrated operation from sample to result	lateral flow device
HIV	paper devices fabricated with cellulose paper and flexible plastic plate	electrochemistry
Neisseria meningitides	versatile paper devices integrated with isothermal amplification	visual fluorescence
Listeria monocytogenes	loop-mediated isothermal amplification (LAMP)-based paper devices	visual fluorescence
Cochlodinium polykrikoides	paper devices based on LAMP	visual fluorescence
Staphylococcus aureus	self-priming paper devices	visual fluorescence
Vibrio parahemolyticus	self-priming paper devices	visual fluorescence
Mycobacterium smegmatis	paper devices combined thermal lysis and isothermal amplification into a single step	visual fluorescence
Bacillus subtilis	a wax-printed cellulose paper device	colorimetry
Salmonella	paper devices integrated with purification, amplification, and on-site detection	colorimetry
Escherichia coli	foldable paper devices with the ability of long-term reagents storage	colorimetry
	paper devices based on isothermal amplification and on-chip detection	visual fluorescence
	paper machine integrated sample preparation and isothermal amplification with end point detection	visual UV/camera
	paper devices integrated extraction, purification, amplification and detection	smartphone/naked eye
	paper devices combined thermal lysis and isothermal amplification	visual fluorescence
bovine infectious reproductive diseases	multiplexed and point-of-care paper-analytical device	visual UV/ smartphone
highly pathogenic strain of porcine reproductive and respiratory syndrome virus (HP-PRRSV)	paper devices fabricated with filter paper and plastic chip	colorimetry

(**Source:** Mao et al., 2020, Environ. Sci. Technol. 2020, 54, 3733−3735



Potential pathways and origins of antiviral drug-resistant viruses through environmental waters.

(Source: Kumar et al., 2020**.** Environ. Sci. Technol. 54, 8503−8505)

Conclusions

- Recent outbreak of a novel coronavirus (COVID-19) has posed a notable public health threat globally.
- WBE has been proven as efficacious approach for tracing the other viral infections.
- Wastewater based epidemiology is an effective approach for prediction of the spread of infection by testing for infectious agents in wastewater.
- The analysis of SARS-Co-V-2 in community wastewater would trace the numbers of potential virus carriers in specific local areas.
- This can be used as an alarming approach for COVID-19 outbreak in a community.
- WBE can also be employed to report efficiency of public health interventions

Thank You

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