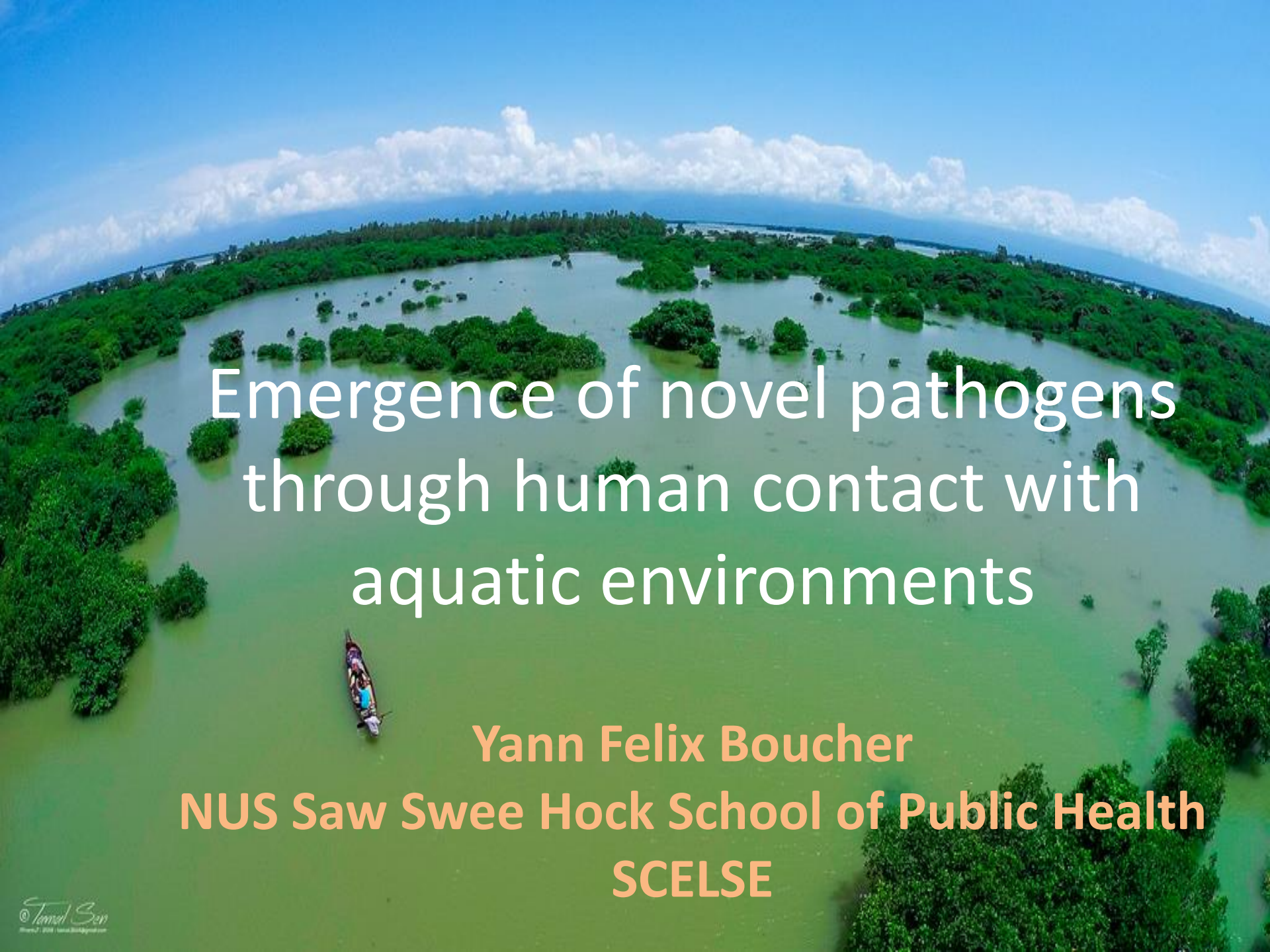
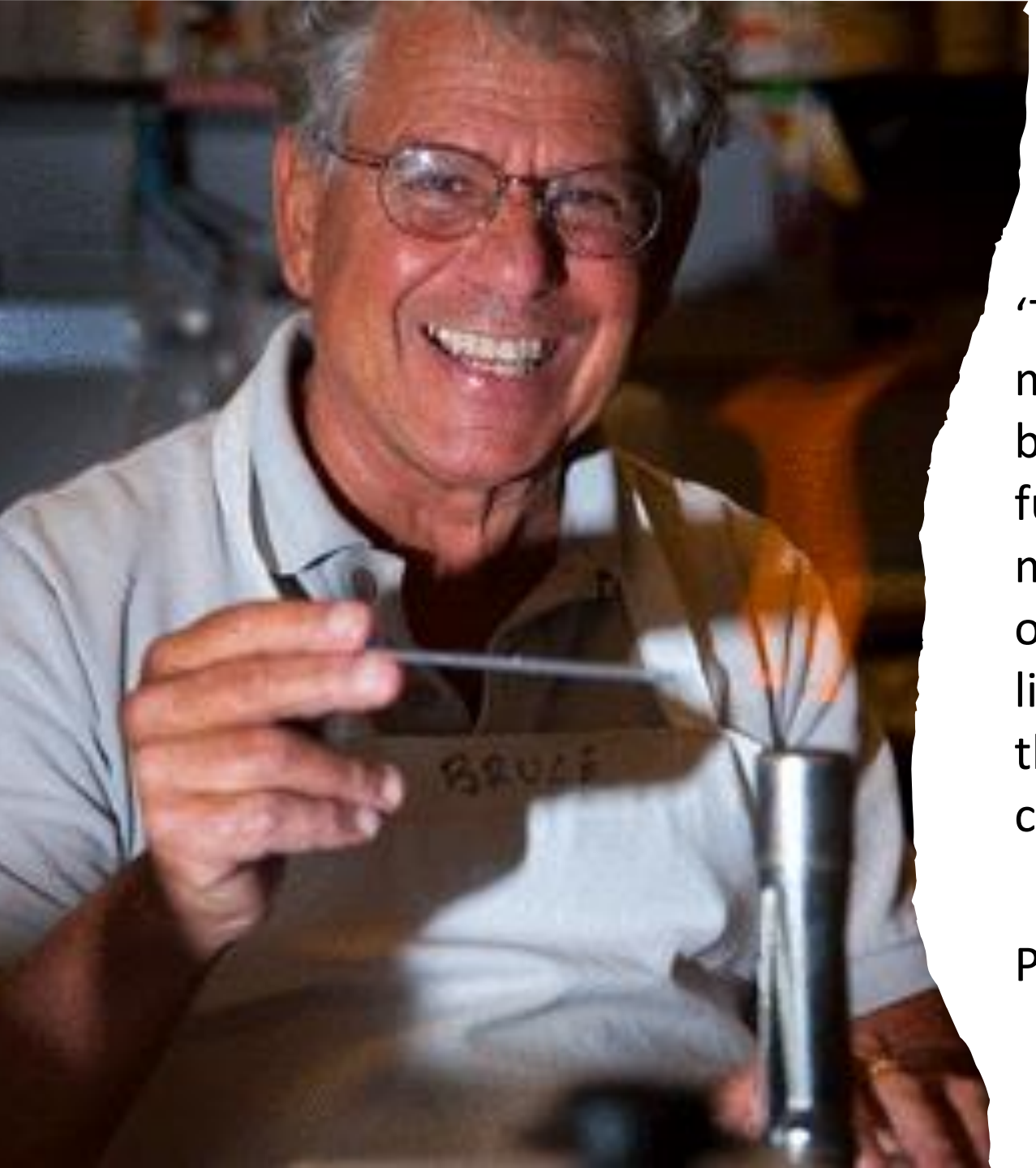


Emergence of novel pathogens through human contact with aquatic environments

Yann Felix Boucher
NUS Saw Swee Hock School of Public Health
SCELSE



‘To pathogenic microparasites (viruses, bacteria, protozoa, or fungi), we and other mammals (living organisms at large) are little more than soft, thin-walled flasks of culture media.

Prof. Bruce Levin

Three ways environmental microbes can be pathogenic to humans

SPECIALIST PATHOGENS: They slowly evolve from an environmental or commensal ancestor and acquire virulence traits that make them adapted to the human host. Humans are an essential part of their life cycle.

Examples: *Vibrio cholerae* O1 El Tor, *Mycobacterium tuberculosis*

GENERALIST PATHOGENS: Their inherent properties make them dangerous to humans, but it is just a coincidental host. They circulate in nature and require hosts for reproduction.

Examples: *Clostridium botulinum, Borrelia burgdorferi (lyme disease), Yersinia pestis*

OPPORTUNISTIC PATHOGENS: A microbe that is not normally pathogenic but can infect a weakened host. These can be human commensals or environmental microbes

Examples: *Pseudomonas aeruginosa*, *Rhizopus* (mucormycosis), *Legionella pneumophila*

Severity of cholera

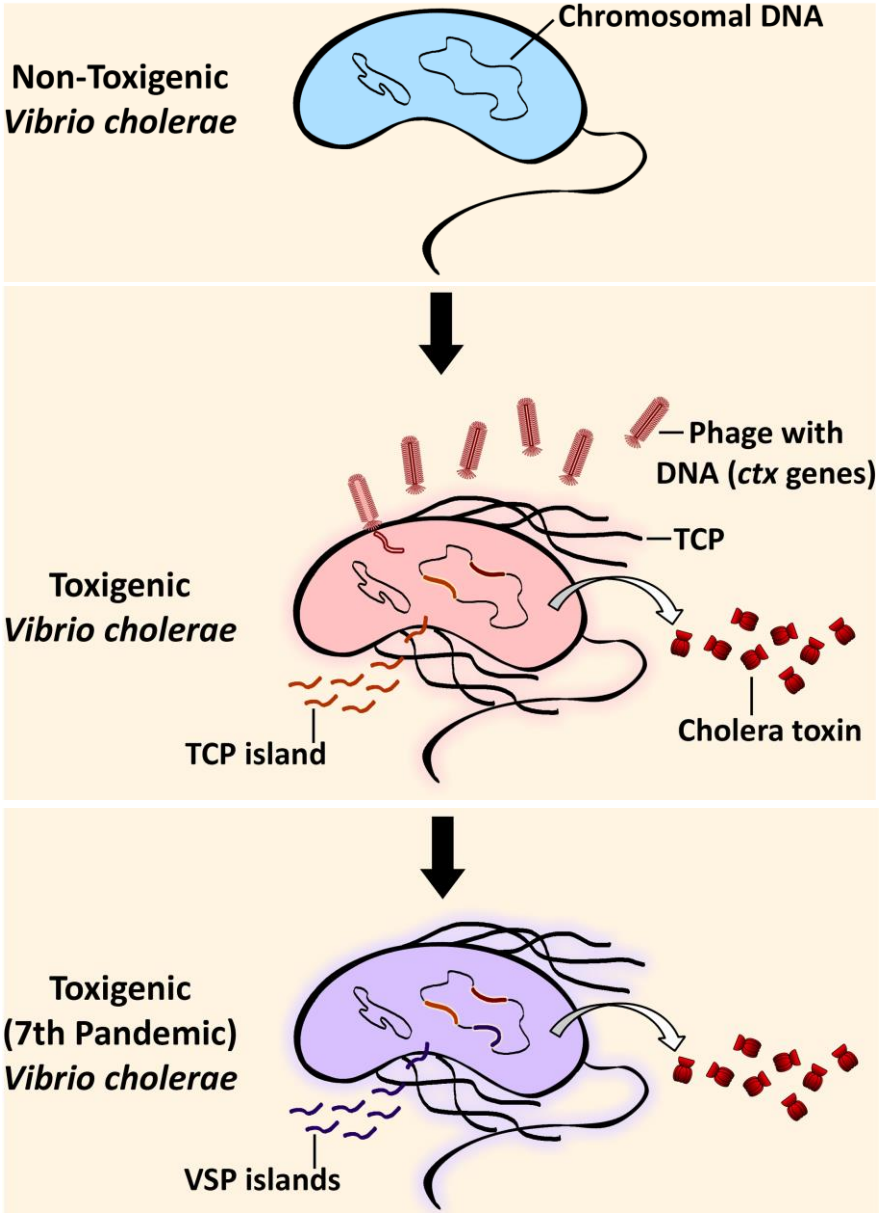
'Two malarias and a Cholera'
Ray Parkin (1943)

- Acute diarrheal disease
- 1.3 to 4.0 million cases every year
- Waterborne disease that follows disasters and wars

A person in the best of health, when he is smitten by the cholera, is in an instant transformed into a corpse…I think it is a disease that begins where other diseases end, with death.

-Francois Magendie, French physician who observed cholera in Tyneside, England, during the second pandemic in 1831

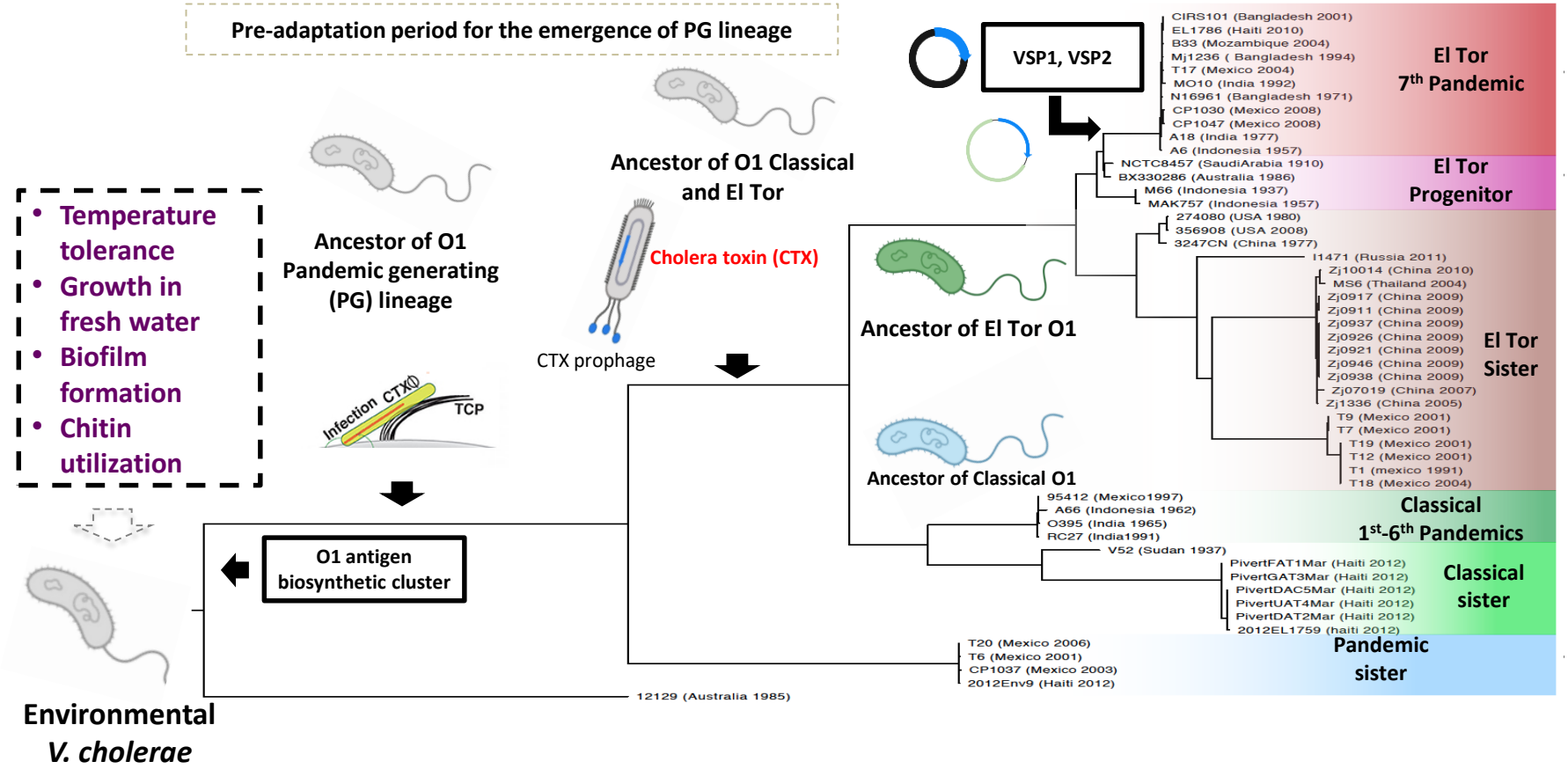
Evolution of toxigenic *V. cholerae*



- > 200 serogroups
- O1 associated with pandemics and has two biotypes
- 1st to 6th pandemics were caused by classical O1
- El Tor O1 is predominant in 7th pandemic cases

(Adapted from Orata *et al,* 2014)

Emergence and evolution of pandemic *Vibrio cholerae*

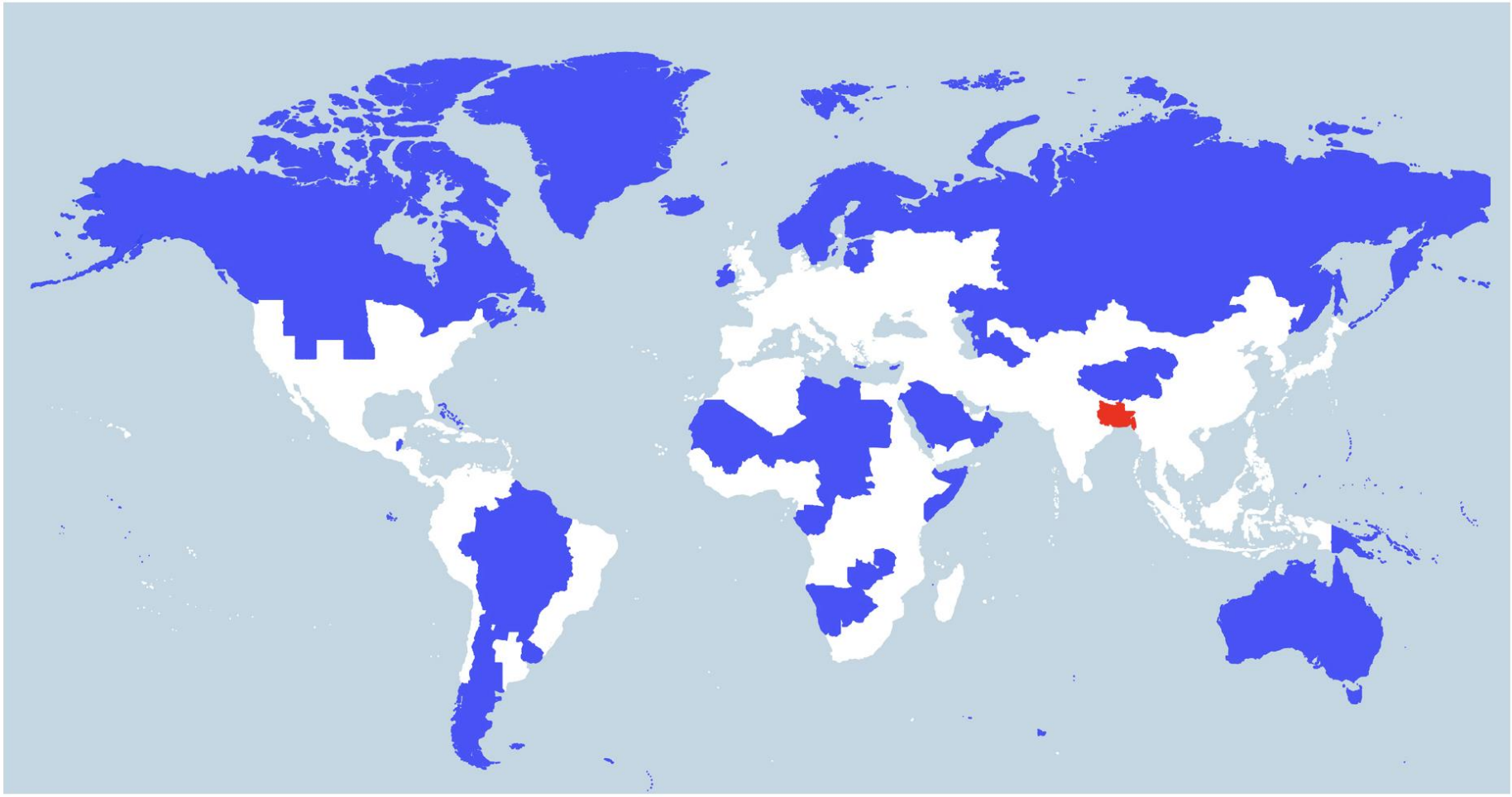


Islam and Boucher, 2017, International Microbiology

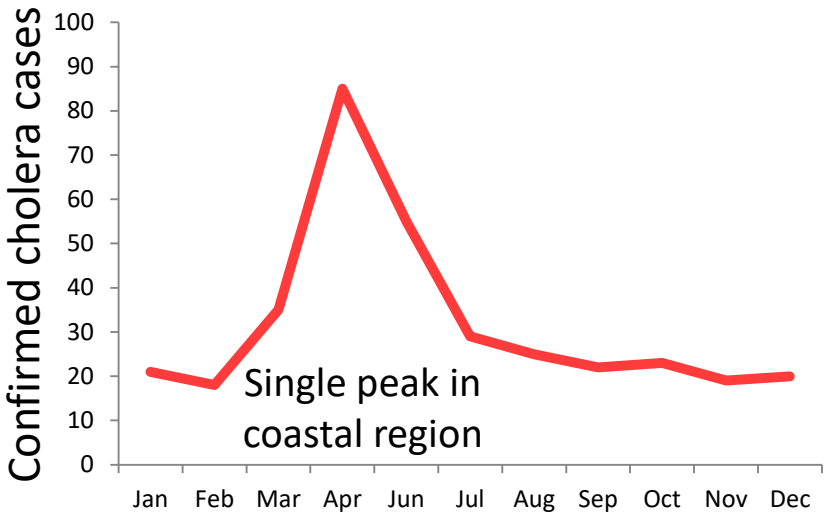
The out-of-the-delta hypothesis: dense human populations in low-lying river deltas served as agents for the evolution of a deadly pathogen

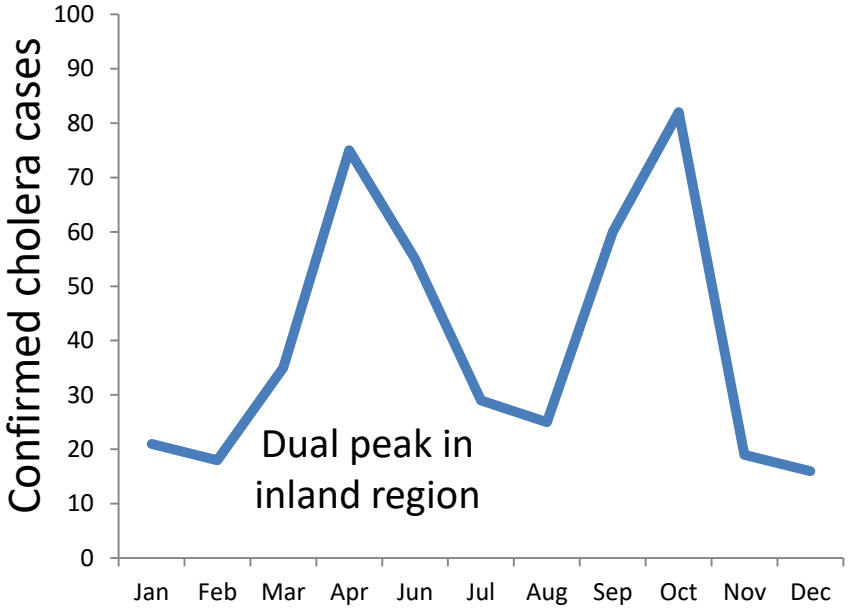
Yan Boucher [1]*, *Fabini D. Orata* [1] *and Munirul Alam* [2]

[1] *Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada,* [2] *Centre for Communicable Diseases, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh*

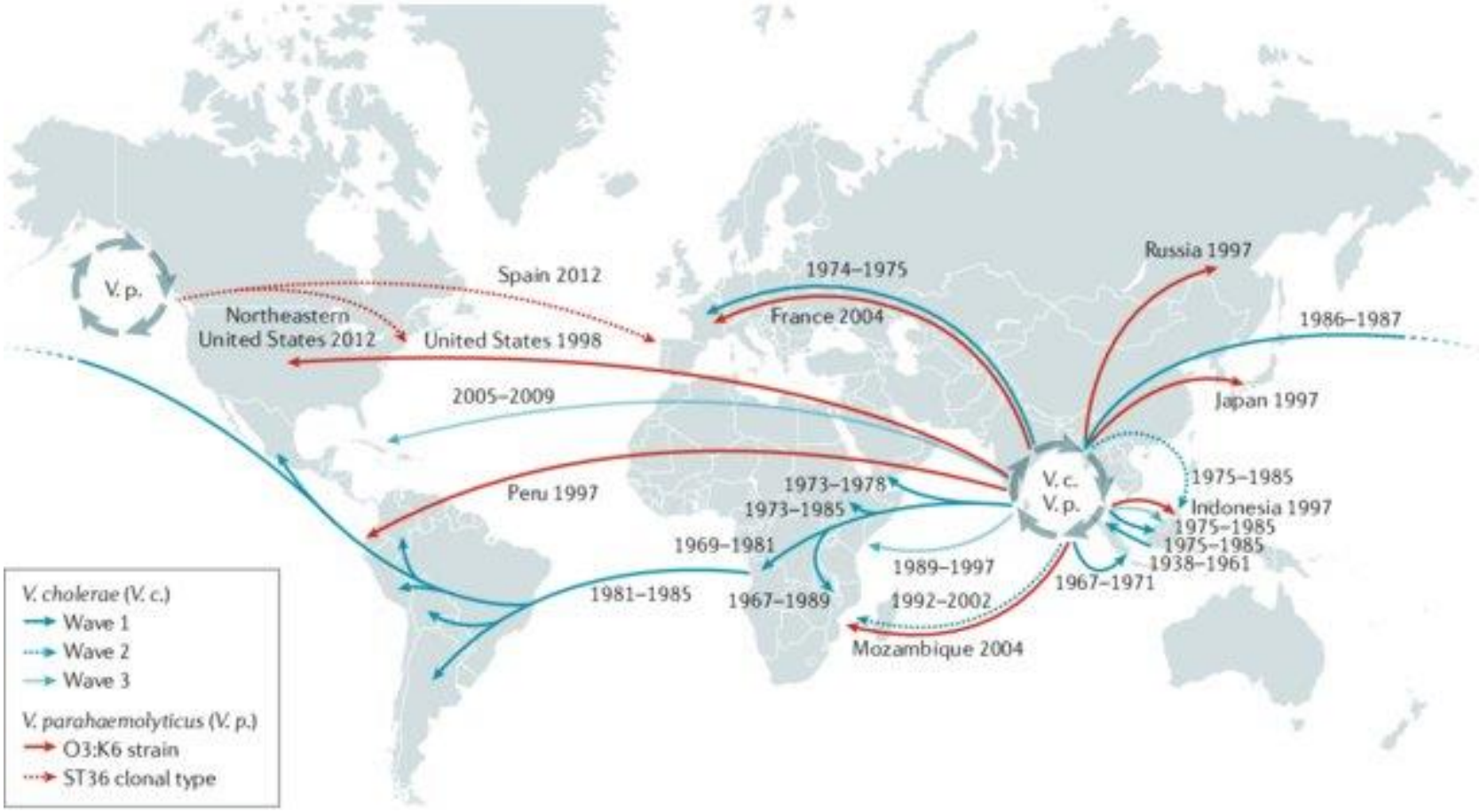


The Bay of Bengal: continual presence of cholera for hundreds of years





The bay of Bengal is the worlds reservoir for cholera variants



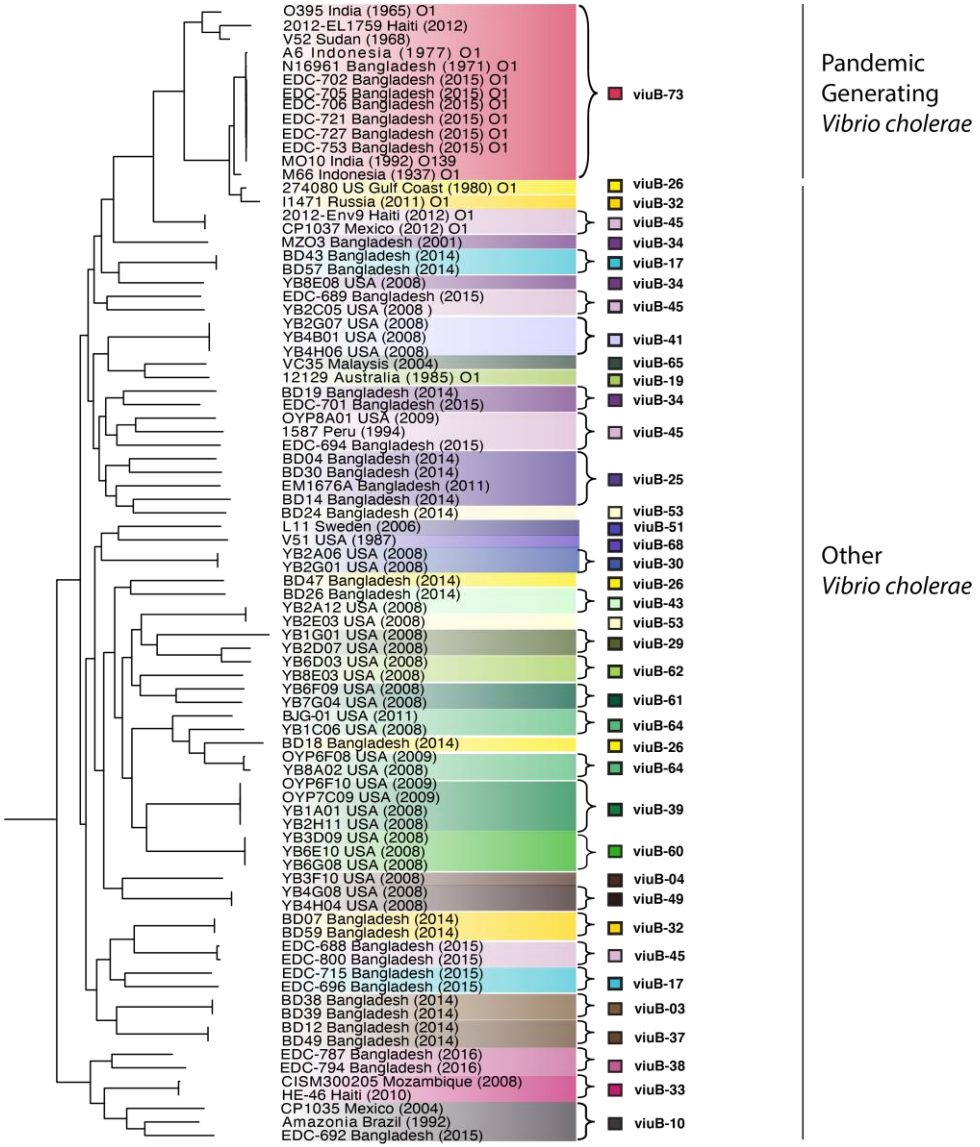
Gene Marker Amplicon Sequencing

Subspecies microbial ecology (*Vibrio cholerae*)

GRAMPS

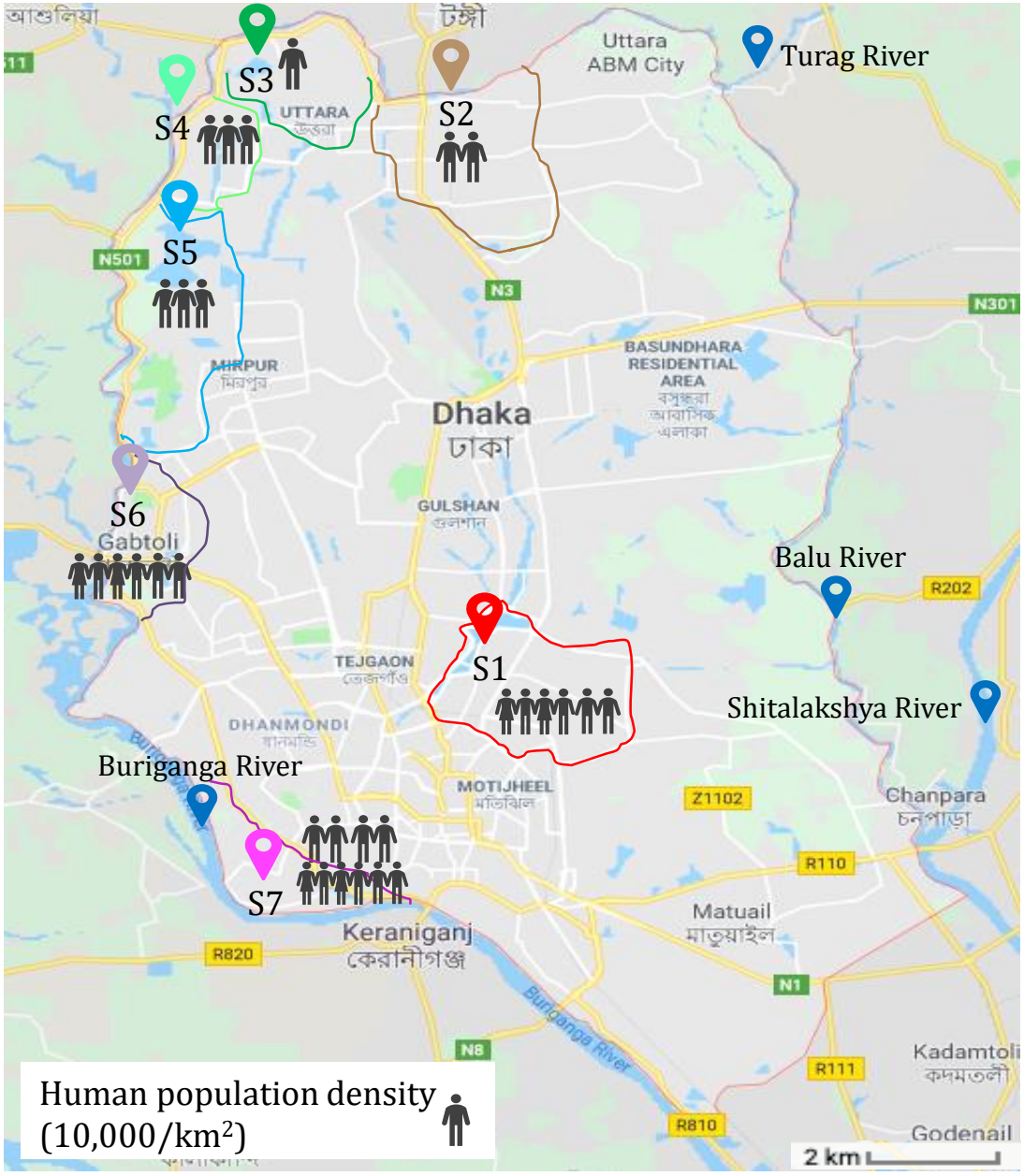
Gene
maRker
Amplicon
Massively
Parallel
Sequencing

maRker:
Vibriobactin utilization protein subunit B (*viuB*) 272bp fragment



Kirchberger & Boucher, Environmental Microbiology 2020

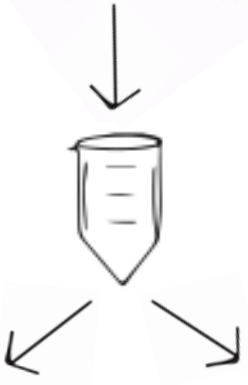
Sampling sites in Dhaka



Sterivex
filter (0.22µm)

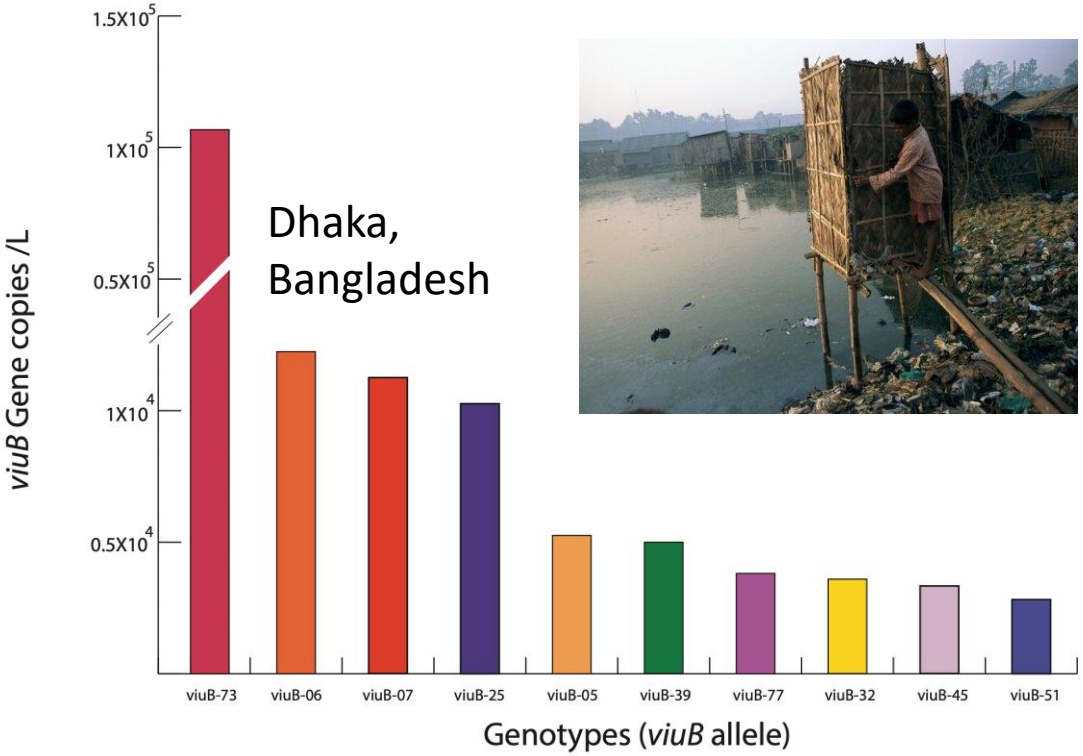
Biomass

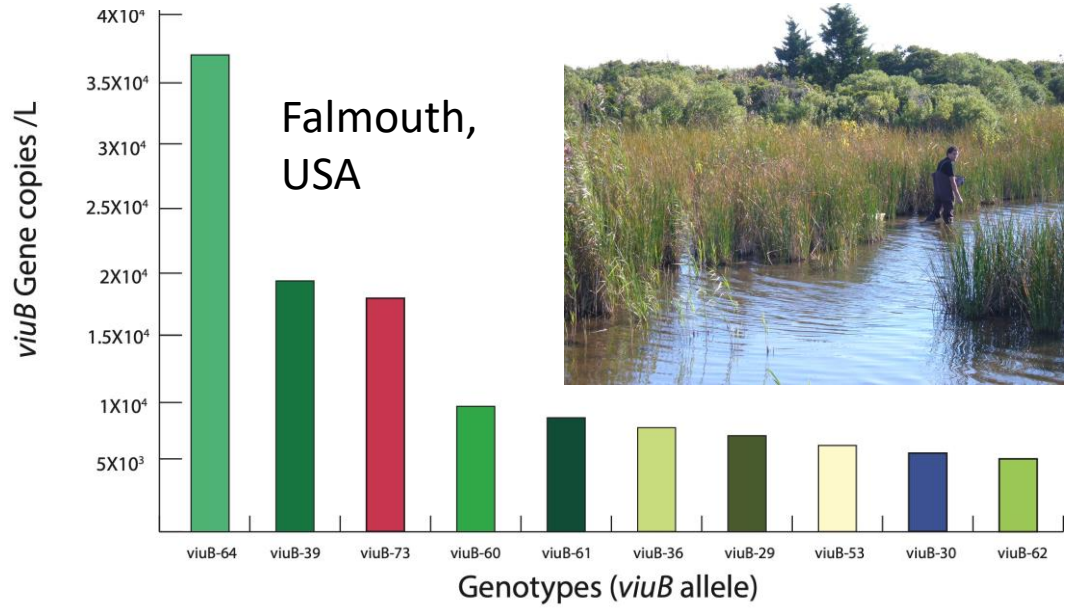
DNA extaction



viuB
Amplicon
sequencing

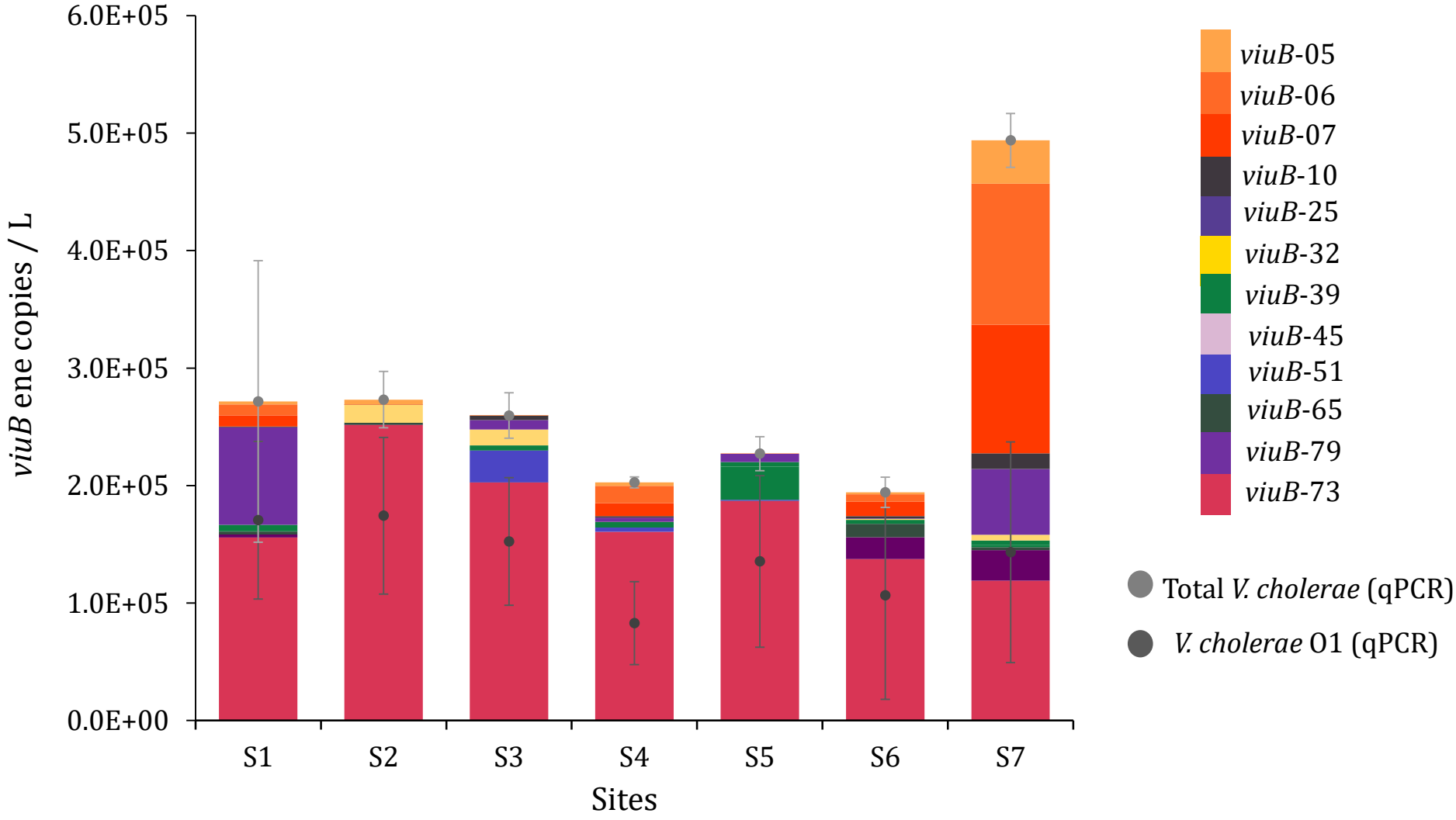
qPCR
-*rfbO1* (*V. cholerae* O1)
-*viuB* (total *V. cholerae*)



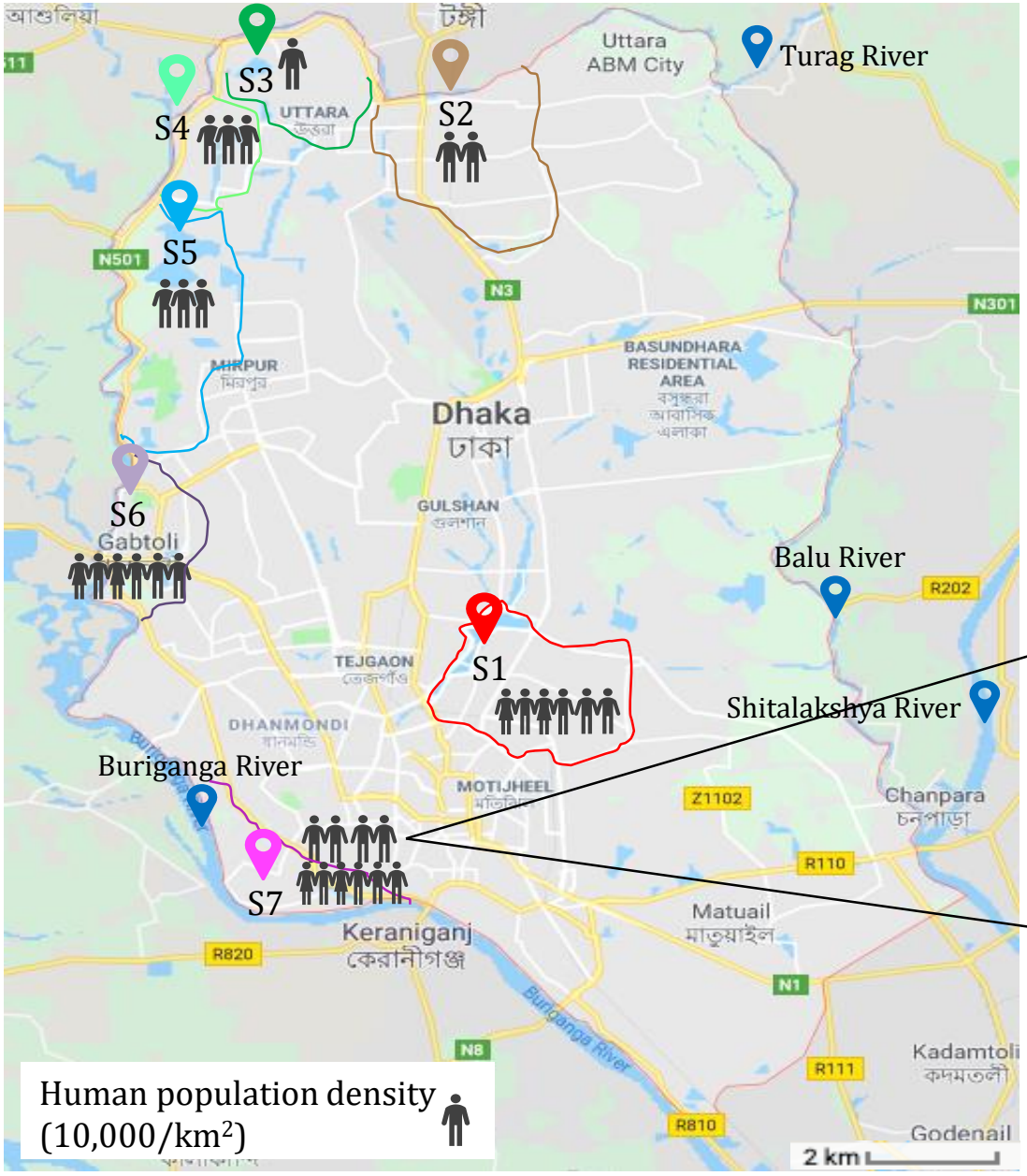


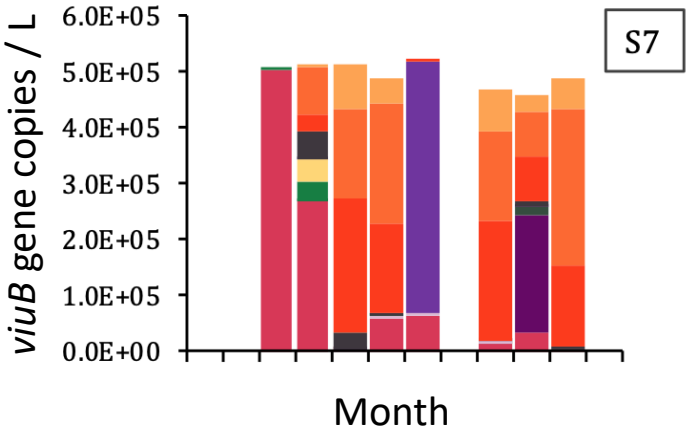
Dhaka has a unique mix of *V. cholerae* genotypes

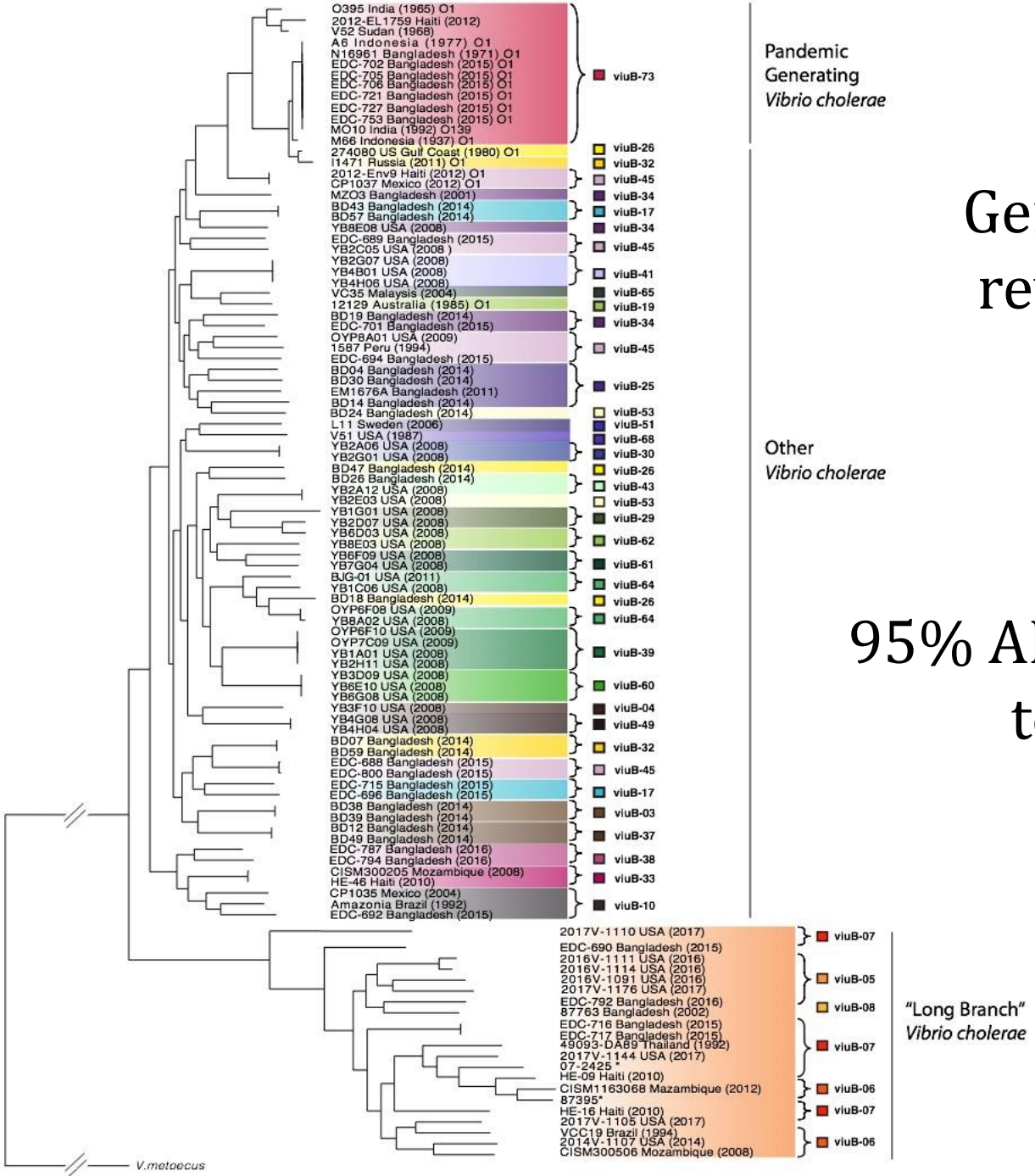
Geographical distribution of *V. cholerae* genotypes in Dhaka



A link to the human gut ?





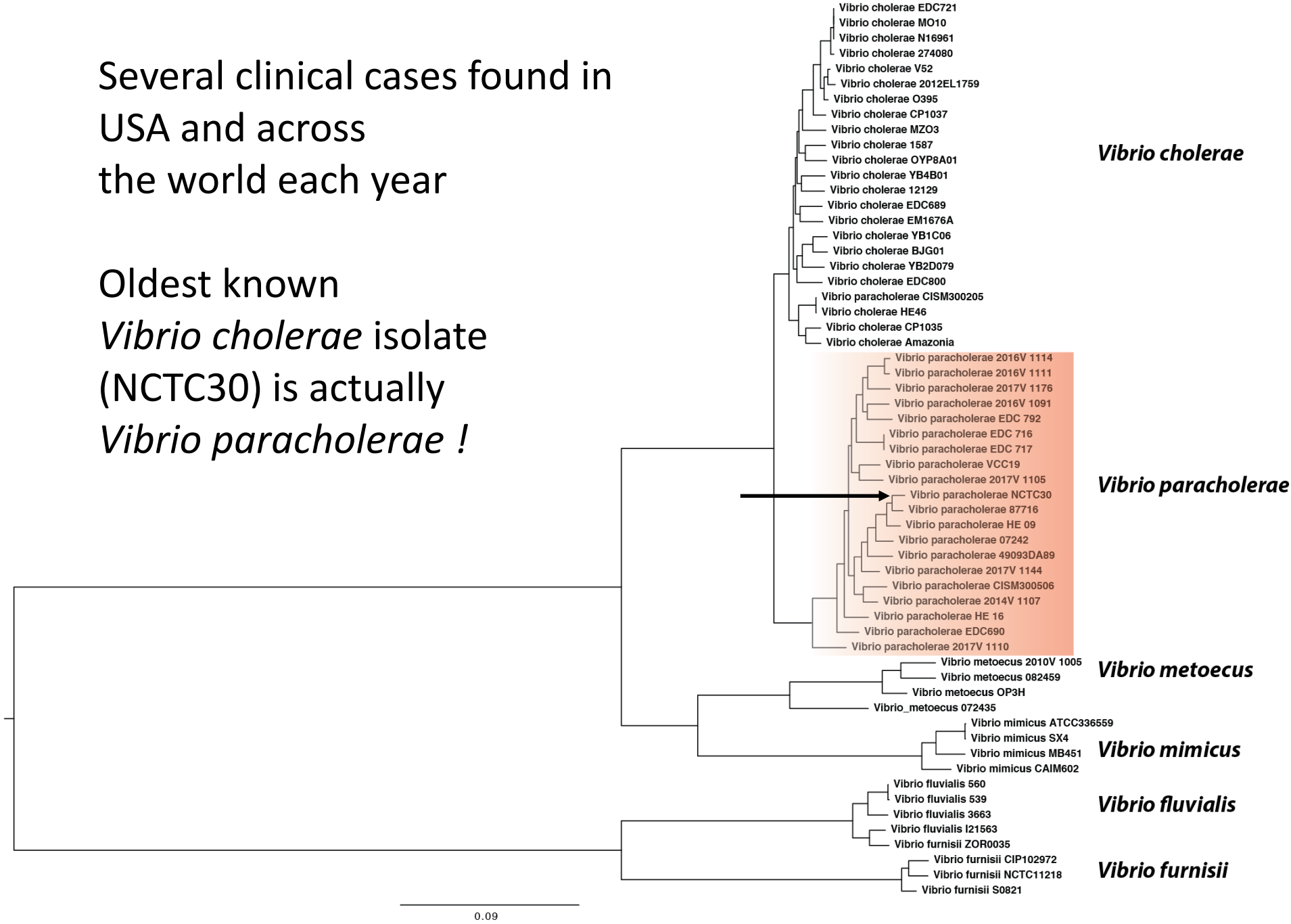


Genome sequencing reveals these novel genotypes are a new species

95% ANI and 69% dDDH to *V. cholerae*

Several clinical cases found in USA and across the world each year

Oldest known *Vibrio cholerae* isolate (NCTC30) is actually *Vibrio paracholerae !*



A bit of history merges with the story…..

Gardner and Venkatraman named the isolate: *Vibrio paracholerae* in 1935!

Vibrio sp. (Gardner & Venkatraman group VI), NCTC 4716.
Vibrio sp. (Gardner & Venkatraman group II), NCTC 8042.
V. eltor NCTC 5395.
V. paracholerae (Gardner & Venkatraman group III), NCTC 30.
V. alcaligenes (Lehmann & Neumann, 1931), NCTC 9239.
V. percolans (Mudd & Warren, 1923), NCTC 1937.
V. proteus (Buchner, 1885), NCTC 8563.
V. cuneatus (Gray & Thornton, 1928), NCIB 8194.
V. cyclosites (Gray & Thornton, 1928), NCIB 2581.
V. neocistes (Gray & Thornton, 1928), NCIB 2582.
Vibrio 01 (Happold & Key, 1932), NCIB 8250.

Strain maintenance

Stock cultures were grown 24 hr. on nutrient agar slopes in loosely closed screw-capped bottles and stored at room temperature with the caps screwed down; fresh cultures were prepared monthly.

World War I in Alexandria Egypt 1916

Soldier in recovery suffered choleraic diarrhea

b4. [redacted] intermediate V. cholerae / V. parahaemolyticus

b4 [redacted] ; 12/10/09 vibrio · cholerae non 01, non-0139.

Genus and species Vibrio sp. Subgroup III Cat. No. 30
Name of strain Martin No. 1. ATCC 14735
Isolated by Lt. Col. C. J. Martin. of Montazah date 1916
Source from stool of Pte. Green, 4th East Lancs. convalescent at Montazah.
Received from Lister Institute London. date March, 1920
Recorded by CS & VJG date 1950 Confirmed by CSB date Feb. 1951
Card check KJS+AX later 3/62 CC Batch 2 11/56 on Batch No. 1.
References in literature Gardner & Venkatraman, 1935. J. Hyg. 35, 262

N4 typed probably O:2 CD for TL 6/11/23

Genus & Species Vibrio sp. Subgroup III Strain Martin No. 1. Cat. No. 30

MORPHOLOGY Medium N Br. ... pH 7·6 T° 30 ... 1 d. — 22. 30. 37. / − ++ + / 4° −
SHAPE Spheres, short rods, long rods, filaments, commas, spirals
SIZE 0.5 x 3-10μ AXIS Straight, curved. mainly curved
SIDES Parallel, bulging, concave, irregular.
ENDS Rounded, truncate, concave, pointed.
ARRANGEMENT Singly, pairs, fours, chains, groups, clusters, bundles, cubical packets, Chinese letters.
REGULARITY Monomorphic, pleomorphic. Club, filaments, branched citron, navicular, fusiform, giants, shadow.
MOTILITY + FLAGELLA Mono-, amphi-, lopho-, peritrichate.
SPORES Spherical, oval; equatorial, sub-, terminal; no bulging.
STAINING Gram -ve Not acid-fast; even, irregular bipolar, barred, beaded. Staining variable. Metachromatic granules.
CAPSULE

COLONY Medium lemco agar pH 7·6 T° 30 3 d.
SIZE 1-3 mm. SHAPE Circular, irregular, radiate, rhizoid.
ELEVATION Effuse, raised, low convex, domed, umbonate.
SURFACE Smooth; fine, medium or coarsely granular; rough; striated; beaten-copper; ringed; papillate; dull; shining.
EDGE Entire, undulate, lobate, crenated, erose, fimbriate, curled, effuse, heaped-up.
COLOUR brown/grey ..Flourescent, iridescent, opalescent, self-luminous.
OPACITY Transparent, translucent, opaque.
CONSISTENCY Butyrous, viscid, friable, membranous.
EMULSIFIABILITY Easy, difficult. Suspensions uniform, granular.
DIFFERENTIATION Centre Periphery
VARIATION

BROTH Medium lemco broth pH 7·6 T° 30 1 d
GROWTH None, scanty. moderate, abundant, profuse.
SURFACE GROWTH Present, absent; slight, moderate, abundant; ring, pellicle, thin, thick, smooth, rough, disintegrates.
TURBIDITY Present, absent; slight, moderate, dense; uniform, granular, flocculent.
DEPOSIT Present, absent; slight, moderate, abundant; powdery, granular, flocculent, viscid Disintegrates yes
ODOUR Absent; resembles (earthy amines).

METABOLIC
Aerobe, facultative anaerobe, anaerobe, microaerophil.
% CO_2 favours, required for growth.
T° range 22 to 37 Optimum 30°C
pH range to Optimum
Pigment greyish brown
Soluble in water, ether, alcohol, $CHCl_3$
Potato
Utilisation of citrate S-K- urea
NH_4 -
KCN -ve

BIOCHEMICAL
Final pH in glucose broth at d.
Indole + Cholera red Weak + (++)
M.R. -ve V.P. -ve NH_3 + H_2S -ve
Nitrates not reduced; nitrogen produced NO_2 not ...
M.B. Catalase +
LITMUS MILK s/acid ... alkaline ...
neutral ... Acid clot
Rennet clot Digestion
Litmus decolourised
Gelatin liquefied + 4
Digestion of serum, egg, meat + 8
7 day broth - Nitrite present

METABOLIC PRODUCTS
Haemolysin for r.b.cs
Leucodin for w.b.cs
Toxin Filterable Antigenic
Action

FERMENTATION
Glucose A
Arabinose -
Xylose -
Galactose SEA-(+)
Mannose A
Lactose SEA(-)
Sucrose A
Maltose A
Trehalose A
Raffinose -
Starch A
Inulin
Glycogen
Dextrin A
Glycerol -
Mannitol A
Dulcitol -
Sorbitol -
Inositol -
Adonitol -
Salicin -
Aesculin

HYDROLYSIS OF
Starch +
Urea Cc. ...
Na hippurate

Blood agar β haemolysis
Gelatin stab Stratiform liquefaction
Meat: gas, foul odour, pink, black, crystals
Resistance: Killed at C. for mins.
Special media N+L ... A ... A
Acriflavine test partially smooth
Growth inhibited by
Growth stimulated by
Essential growth factors
ANTIGENIC STRUCTURE
PHAGE TYPE
PATHOGENICITY

X. Dried. 23.10.50
Batch No. 1. 26.1.51 3. 24-7-62
2. 9.11.56 4. 3.4.85

oxidase +
gluconate −
malonate −
PA −
MacConkey · NLF
LV no opacity
decarboxylases A - L + O +

Shannon M 8139

Another historical role for *V. paracholerae:* The rise of *V. cholerae* O139

IDCP

MAY–JUNE 1994

EDITORS' FORUM

VOL. 3, NO. 3

THE EIGHTH CHOLERA PANDEMIC

A person in the best of health, when he is smitten by the cholera, is in an instant transformed into a corpse!. . . I think it is a disease that begins where other diseases end, with death.

—François Magendie, French physician
who observed cholera in Tyneside, England,
during the second pandemic in 1831

Not yet finished with the seventh pandemic of cholera, which began in 1961 and finally reached the Americas in an outbreak of unparalleled ferocity in Peru just 3 years

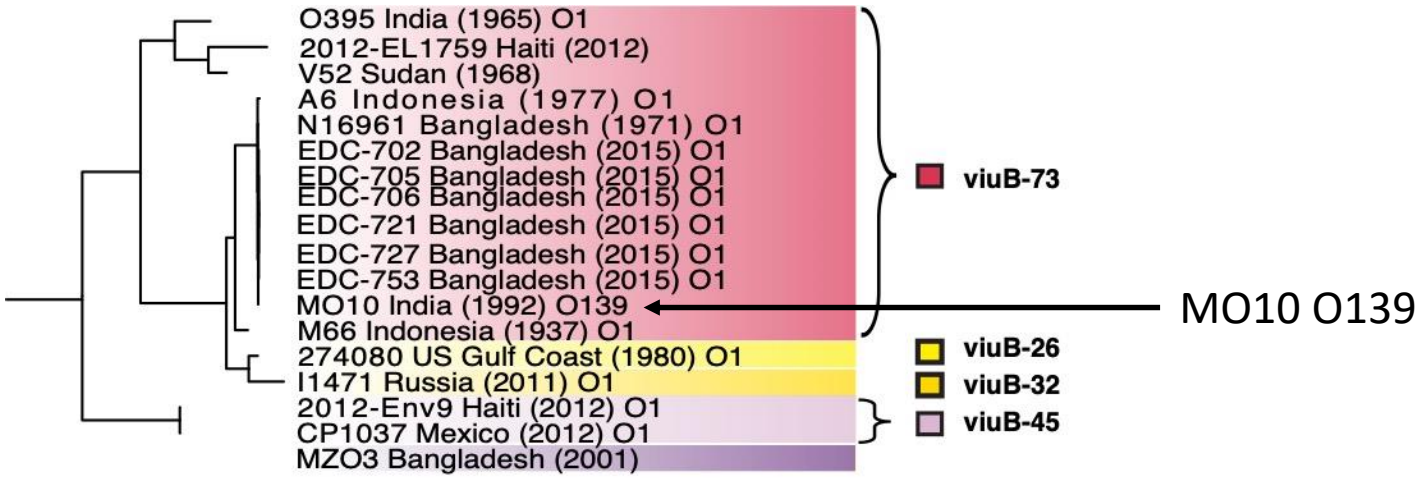
dead or past recovery within less than an hour." These outbreaks, however, did not spread beyond the subcontinent. (For more details on the history of cholera, see Pollitzer

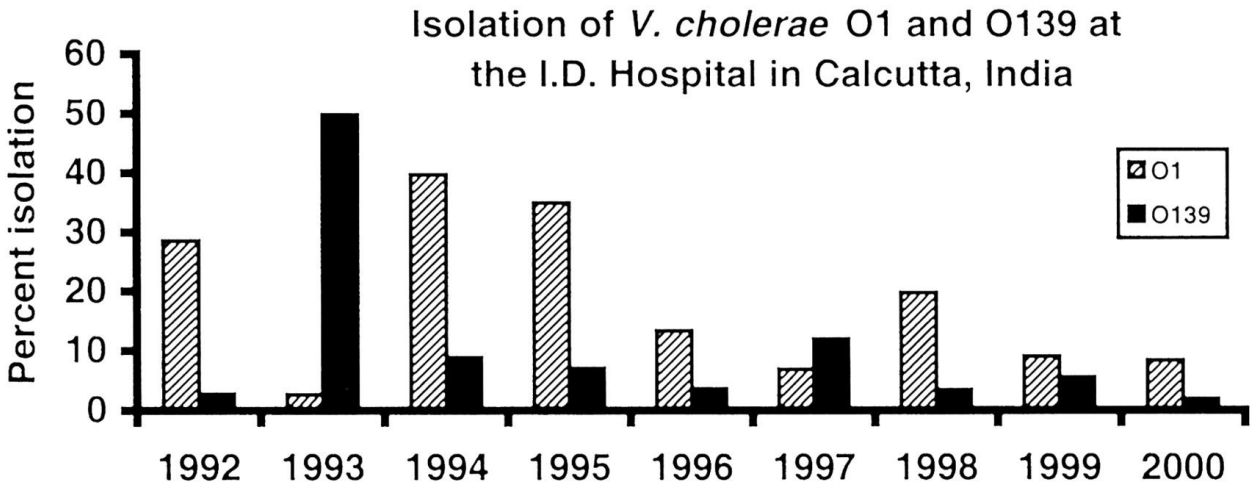
pandemic in the 1890s, which was recorded in the celebrated novel, *Love in the Time of Cholera*, by Gabriel García Márquez.

The current (seventh) pandemic broke the traditional pattern by not beginning in Bengal, where all previous pandemics had their start, but rather in the Celebes, Indonesia, during 1961. The epidemic strain also was different from the classical *Vibrio cholerae* strains of previous pandemics. The new biotype was identified as *el tor*, a strain that had originally been isolated at the town of El Tor on the Sinai peninsula in 1886. The *el tor* isolates of the seventh pandemic differed slightly from the previous *el tor* strains by lacking a hemolysin; they were otherwise similar, and they produced abundant quantities of cholera toxin.

When I arrived in Calcutta during

O139 is a 7th pandemic strain with new antigens





V. paracholerae played a role in the rise of novel pathogenic variant of *V. cholerae* (O139)

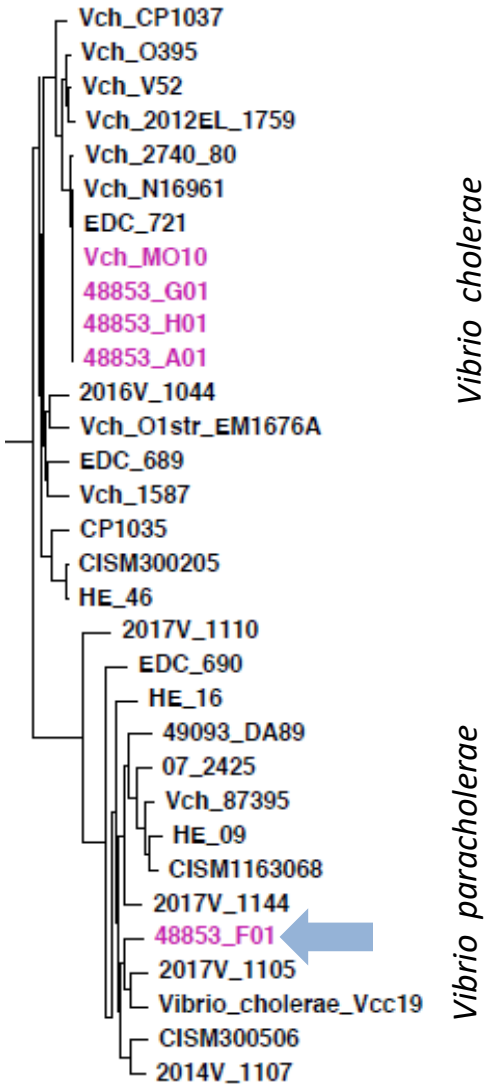
PLOS NEGLECTED TROPICAL DISEASES

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Vibrio cholerae Serogroup O139: Isolation from Cholera Patients and Asymptomatic Household Family Members in Bangladesh between 2013 and 2014

Four *V. cholerae* O139 isolates from clinical samples



V. cholerae O139

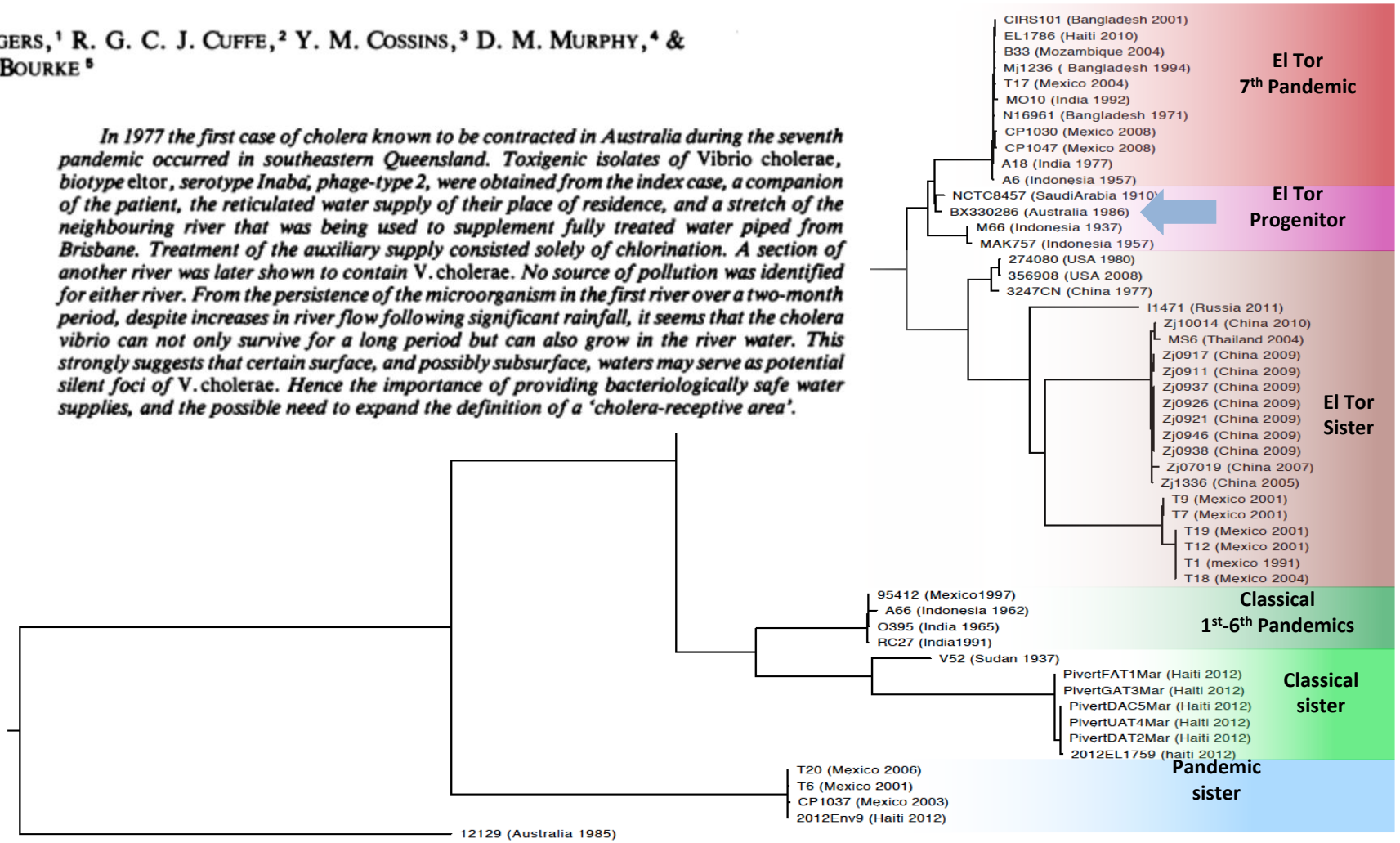
Where will the next variant come from ?

Bulletin of the World Health Organization, 58 (4): 665-669 (1980)

The Queensland cholera incident of 1977. 2. The epidemiological investigation*

R. C. ROGERS,[1] R. G. C. J. CUFFE,[2] Y. M. COSSINS,[3] D. M. MURPHY,[4] & A. T. C. BOURKE[5]

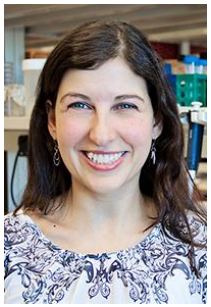
In 1977 the first case of cholera known to be contracted in Australia during the seventh pandemic occurred in southeastern Queensland. Toxigenic isolates of Vibrio cholerae, *biotype* eltor, *serotype Inaba, phage-type 2, were obtained from the index case, a companion of the patient, the reticulated water supply of their place of residence, and a stretch of the neighbouring river that was being used to supplement fully treated water piped from Brisbane. Treatment of the auxiliary supply consisted solely of chlorination. A section of another river was later shown to contain* V. cholerae. *No source of pollution was identified for either river. From the persistence of the microorganism in the first river over a two-month period, despite increases in river flow following significant rainfall, it seems that the cholera vibrio can not only survive for a long period but can also grow in the river water. This strongly suggests that certain surface, and possibly subsurface, waters may serve as potential silent foci of* V. cholerae. *Hence the importance of providing bacteriologically safe water supplies, and the possible need to expand the definition of a 'cholera-receptive area'.*



Thank You …


Dr. Tania Nasreen


Dr. Munirul Alam


Prof. Stephanie Yanow

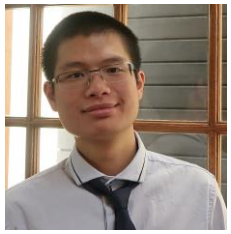

Dr. Cheryl Tarr (CDC)


Dr. Paul Kirchberger


Nora Hussain


ICDDR,B Team

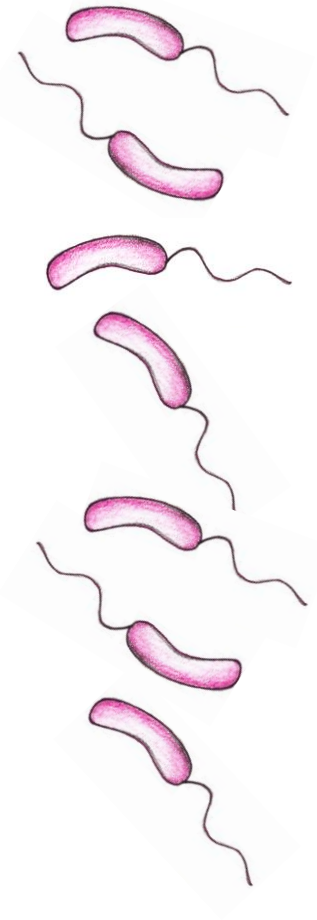

Dr. Fabini Orata


Kevin Liang


Md. Tarequl Islam


Dr. Rebecca Case

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION




How well can you tackle the pandemic?
LOCKDOWN!
A card game

Capital Gains Studio

0

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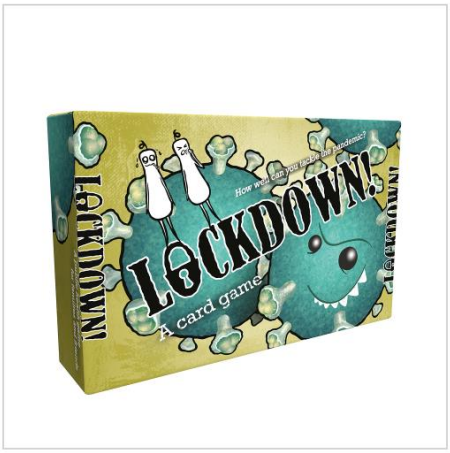
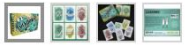
Home / Lockdown!





Lockdown!

SKU: NUS-LD

~~S$25.00~~ S$20.00

Quantity

1

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Lockdown! is an engaging and exciting educational card game aimed at raising public awareness about the Covid-19 pandemic from a public health perspective.

The game was created by two researchers at the National University of Singapore: Associate Professor Yann Boucher from the Saw Swee Hock School of Public Health and Dr Anna Szücs from the Yong Loo Lin School of Medicine.

In Lockdown! participants take on the role of governments and implement various public health measures to save the most Covid-19 patients in their countries. It's a fun, informative and competitive game that can be played individually or in teams of two among family and friends.

Proceeds from Lockdown! will go to The Red Pencil (Singapore).

Pre-order now to get a 20% discount!

Game delivery is expected to be in end Dec 2021.

https://www.capitalgainsgroup.com/product-page/lockdown-an-educational-card-game