

The background of the slide features a close-up photograph of several mussels. The shells are dark, almost black, with prominent ridges and a metallic sheen. The mussels are clustered together, and the lighting creates highlights on their surfaces, giving them a three-dimensional appearance. The overall tone is somewhat dark and moody.

Vibrios, Hepatitis A & Norovirus

Dr Craig Baker-Austin, Cefas

Food and Water Microbiology Proficiency Testing (PT)

International Virtual Meeting, 12th October 2022

Microbial risks in the marine environment

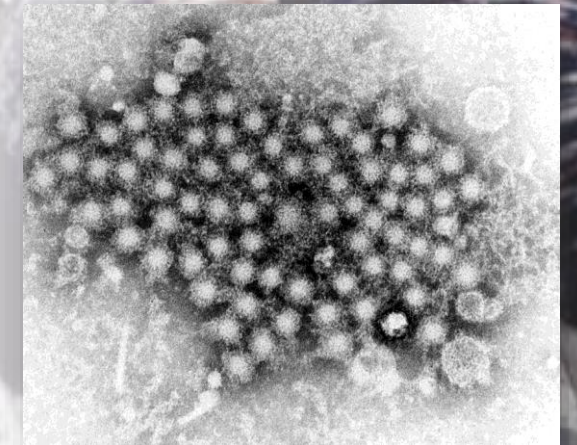
A wide variety of pathogen risks are present in the marine environment. For example, faecal contamination of water can introduce a variety of pathogens, including bacterial, viral, protozoan agents.

Introduced agents include viruses such as enteroviruses, poliovirus, hepatitis A, norovirus and rotavirus. Bacterial pathogens include *Salmonella*, *Campylobacter*, *Shigella*, *Vibrio cholerae* O1/O139 and *E. coli*.

Naturally occurring agents include certain *Vibrio* species as well as harmful algal biotoxin (HABs) producing organisms. Many are also widespread in marine waters worldwide.

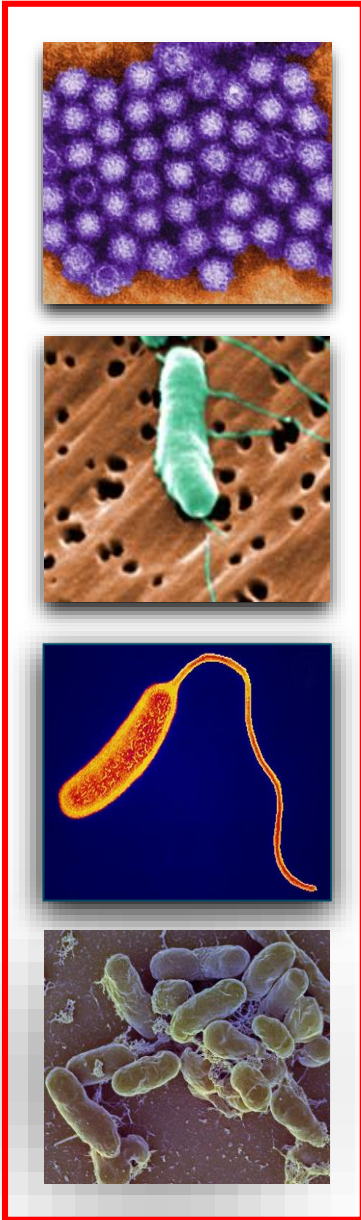


Vibrio cholerae – the causative agent of cholera

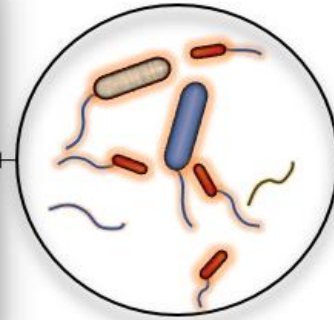


Hepatitis A virus

Shellfish (and water) are an effective vehicle in disease transmission



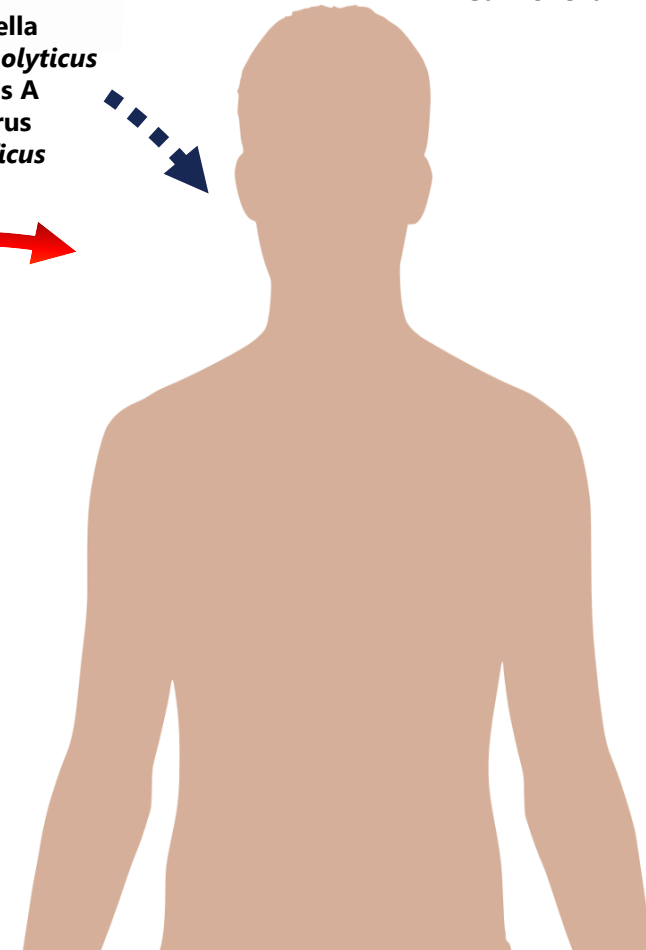
Salmonella
V. parahaemolyticus
Hepatitis A
Norovirus
V. vulnificus



V. cholerae
Norovirus
Hepatitis A
Rotavirus
Astrovirus
Salmonella
E. coli
Shigella



V. cholerae
Hepatitis A
Norovirus
Salmonella

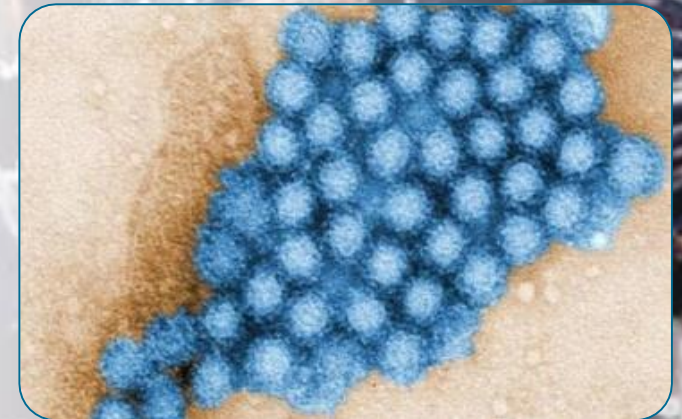
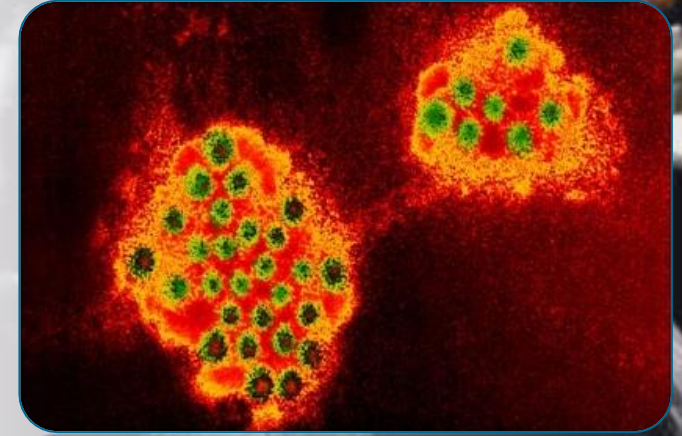


Shellfish and disease risk

Shellfish represent an established route of transmission for a range of important human pathogens. First outbreaks epidemiologically-linked to shellfish consumption emerged in the 19th Century.

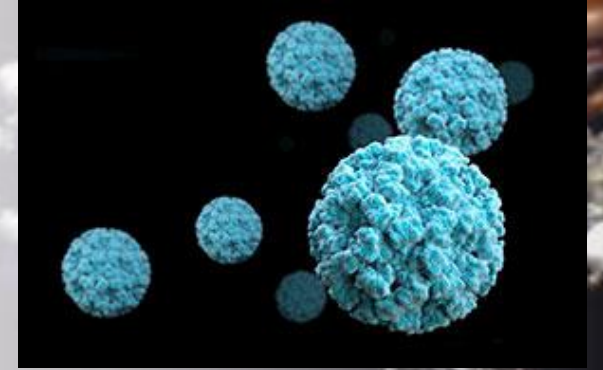
Bivalve filter feeding shellfish species such as oysters, mussels and clams very efficient at filtering and concentrating bacterial, viral and algal toxins. Studies have shown that bivalve shellfish can concentrate microbial pathogens >100 times the concentrations found in overlying waters.

Outbreaks often not trivial: Hepatitis A outbreak in China in the early 1990s was responsible for almost 300,000 cases – one of the largest foodborne outbreaks ever reported.



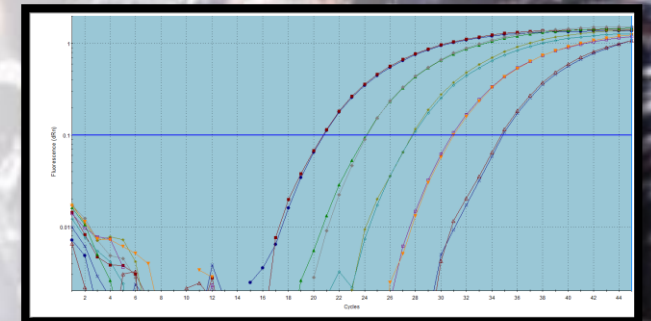
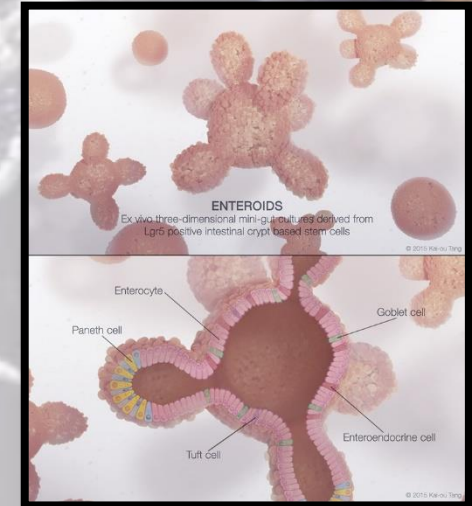
Viruses in shellfish - the problems

- Viruses (esp. norovirus and hepatitis A virus; HAV) recognised as principle cause of illness following shellfish consumption worldwide.
- Several outbreaks of norovirus from shellfish reported in UK annually. Incidents with HAV rarer but can occur.
- Current risk assessment and management world-wide relies on faecal indicators (*E. coli* in EU).
- Bivalve shellfish causing outbreaks are frequently compliant with regulatory standards. Methods for virus detection are an important risk management tool.



Detection of viruses in shellfish

- A number of publications of culture of norovirus in last 10 years. Methods difficult, expensive, require very high starting levels. Propagation of HAV from shellfish also problematic.
- No realistic short- to mid-term prospect of culture methods for shellfish.
- Detection of virus particles using microscopy or virus coat proteins using immunology (e.g. ELISA) currently lacks sensitivity required for low virus levels in shellfish – no reports of successful application to “real” food samples.
- Identification of virus in shellfish currently only possible through “molecular methods” - specific detection of viral nucleic acid (PCR). Current methodology uses quantitative real-time RT-PCR.



Norovirus: ISO 15216

- Norovirus is the most important cause of illness associated with seafood consumption. Globally most common pathogen associated with seafood-associated illness.
- International standard method for determination of viruses in foods (incl. shellfish) using RT-qPCR developed by expert WG led by Cefas scientists.
- ISO 15216 published as a two-part Technical Specification in 2013.
- International method validation programme led by Cefas, 2012-2015. Fully ISO published 2019.
- First virus standard method of its kind in the world.

INTERNATIONAL
STANDARD

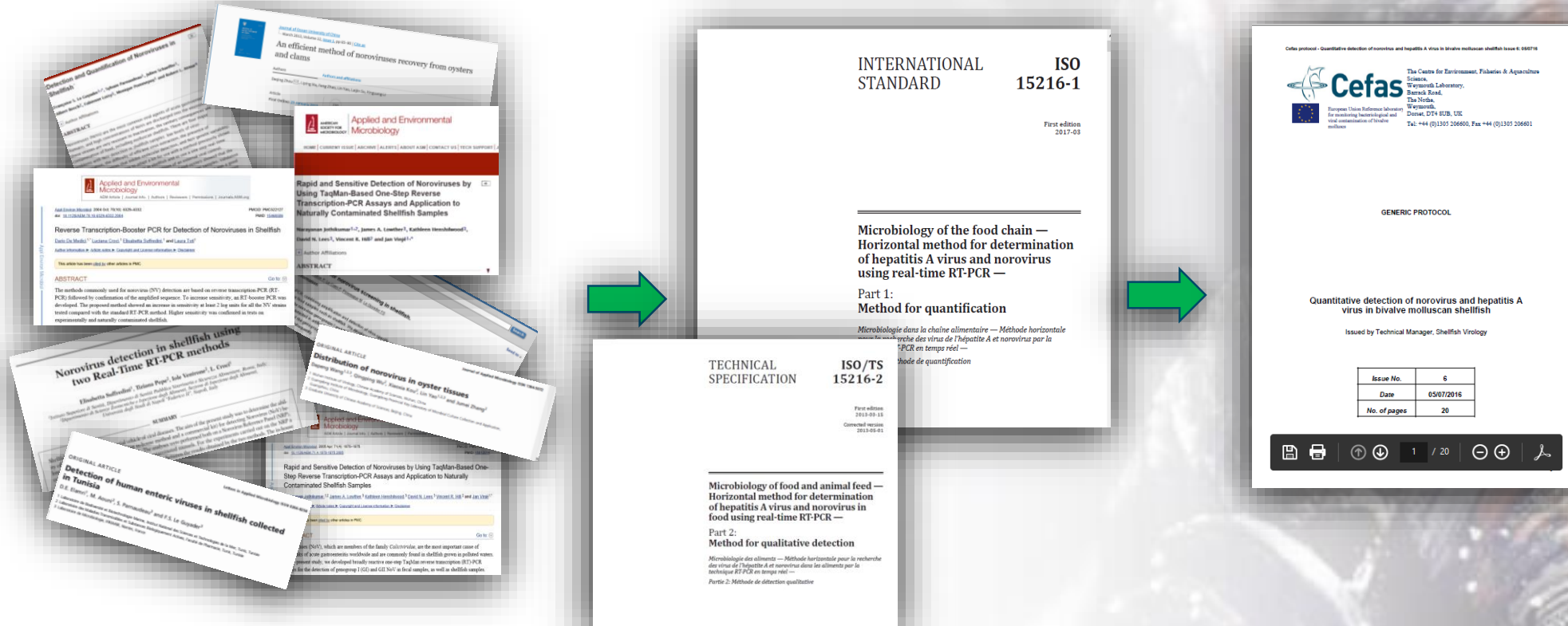
ISO
15216-1

First edition
2017-03

**Microbiology of the food chain —
Horizontal method for determination
of hepatitis A virus and norovirus
using real-time RT-PCR —**

Part 1:
Method for quantification

Viruses – a standardisation story, from 30 to 1 method



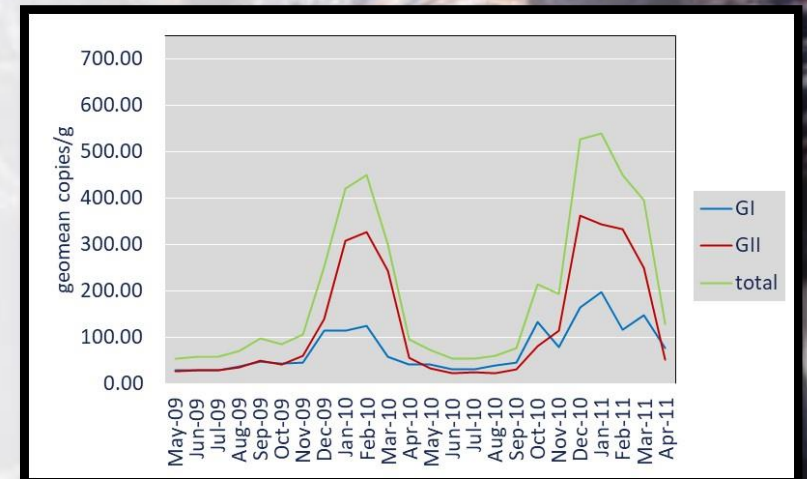
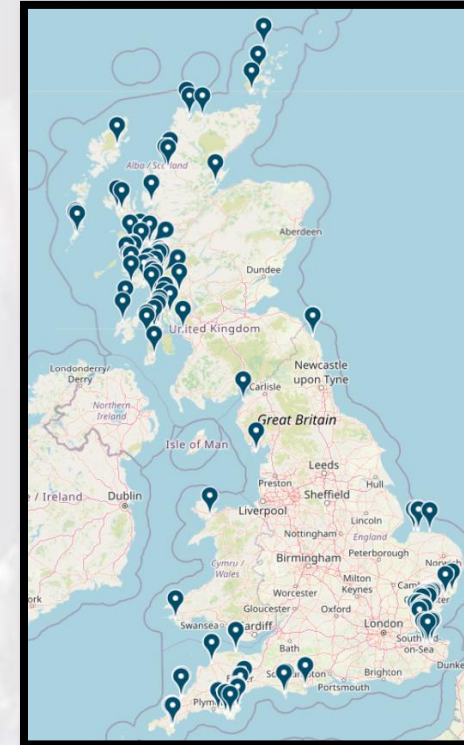
TAG 4, 30+ participants, at least 13 countries, 10 years, 1 ISO!

- 1) 30+ published studies on method
- 2) ISO standard
- 3) Lab protocol

This work took over a decade, and involved massive international efforts

Survey impacts

- Recent surveys led by Cefas (or as a international partner have shown):
 - 11% (European dispatch centres)
 - 35% (European production areas)
 - 69% (UK retail sites)
 - 76% (UK production areas)
- Fit for purpose method now applicable for environmental and food testing.
- **Impact:** First and only virus method of its kind, with direct potential ramifications for risk assessment and future legislation, possible impacts on trade.



HAV and shellfish – a rare issue in the UK?

- HAV is transmitted by the faecal-oral route.
- HAV is an uncommon infection in the UK and other high-income countries. In 2018, Public Health England reported 452 laboratory-confirmed cases of hepatitis A infection in England and Wales.
- HAV rarely detected in UK shellfish.
- In 2012 we became aware of a cluster of cases of HAV in the Netherlands and UK linked by mussels. Likely cause was an infected individual(s) shedding HAV into mussel production area, which led to outbreak.

SURVEILLANCE AND OUTBREAK REPORT

International linkage of two food-borne hepatitis A clusters through traceback of mussels, the Netherlands, 2012

I.L. Boxman¹, I. Verhoef², H. Vennema², S. Ngui³, J.H. Friesema⁴, C. Whiteside⁵, D. Lees⁶, M. Koopmans⁷

1. Laboratory for Food and Food Safety, Food and Consumer Product Safety Authority (NVWA), Wageningen, the Netherlands
2. Laboratory for Infectious Diseases and Screening, National Institute of Public Health and the Environment (RIVM), Bilthoven, the Netherlands
3. Virus Reference Department, Microbiology Services Division - Colindale, Public Health England, London, United Kingdom
4. Epidemiology and Surveillance Unit, Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, the Netherlands
5. Public Health Wales, United Kingdom
6. Centre for Environment, Fisheries and Aquaculture Science, Weymouth, United Kingdom
7. Department of Viroscience, Erasmus MC, Rotterdam, the Netherlands

Correspondence: Ingeborg L.A. Boxman (l.a.boxman@nvwa.nl)

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Boxman I, Verhoef I, Vennema H, Ngui S, Friesema J, Whiteside C, Lees D, Koopmans M. International linkage of two food-borne hepatitis A clusters through traceback of mussels, the Netherlands, 2012. *Euro Surveill*. 2016;21(3):pii=30113. DOI: <http://dx.doi.org/10.2807/1560-7917.ES.2016.21.3.30113>

Article submitted on 01 December 2014 / accepted on 04 May 2015 / published on 21 January 2015

This report describes an outbreak investigation starting with two closely related suspected food-borne clusters of Dutch hepatitis A cases, nine primary cases in total, with an unknown source in the Netherlands. The hepatitis A virus (HAV) genotype 1A sequences of both clusters were highly similar (459/460 nt) and were not reported earlier. Food questionnaires and a case-control study revealed an association with consumption of mussels. Analysis of mussel supply chains identified the most likely production area. International enquiries led to identification of a cluster of patients near this production area with identical HAV sequences with onsets predating the first Dutch cluster of cases. The most likely source for this cluster was a case who returned from an endemic area in Central America, and a subsequent household cluster from which treated domestic sewage was discharged into the suspected mussel production area. Notably, mussels from this area were also consumed by a separate case in the United Kingdom sharing an identical strain with the second Dutch cluster. In conclusion, a small number of patients in a non-endemic area led to geographically dispersed hepatitis A outbreaks with food as vehicle. This link would have gone unnoticed without sequence analyses and international collaboration.

Introduction

Hepatitis A virus (HAV) is a faecal-orally transmitted pathogen causing acute self-limiting hepatitis. Risk factors for infection include exposure to infected persons, contact with faecally contaminated surfaces, food or water. The incidence of hepatitis A in industrialised countries has decreased due to improved sanitary and living conditions. The decreasing immunity in younger

age groups has led to increasing age and severity of first infection [1]. Hepatitis A could therefore re-emerge in regions such as North America or western Europe, where it is not endemic, affecting mostly adults, with more severe course of infection [1]. In the Netherlands the incidence rate for hepatitis A in 2010 was 1.3 cases per 100,000 population [2]. Hepatitis A is a notifiable disease in the Netherlands. Cases, including routine demographic and epidemiological data, are reported according to standardised national criteria [3] by regional public health services using Osiris, a national electronic registration system for infectious diseases hosted by the National Institute for Public Health and the Environment (RIVM). Surveillance is intensified for cases with no travel history to endemic countries and an unknown source of infection. For these cases, sequences are actively collected and additional hypothesis-generating (trawler) questionnaires are routinely administered, aiming to identify a potential common (food-borne) source of infection [2-4].

Recently, food-borne transmission of HAV has been implicated in several multinational outbreaks or involving multiple states in the United States (US), occurring in rapid succession in 2013 and 2014 [5-8]. Such diffuse outbreaks that are characterised by cases that are geographically or temporally dispersed benefit from combining epidemiological data with viral sequence data. Finding the source is challenging due to the long incubation period of two to six weeks [9]. Recall can be assisted by the use of structured questionnaires including known risk factors or risk products [3,4]. Absolute confirmation of a food source is rare, in part due to difficulties with detection of the viral RNA in

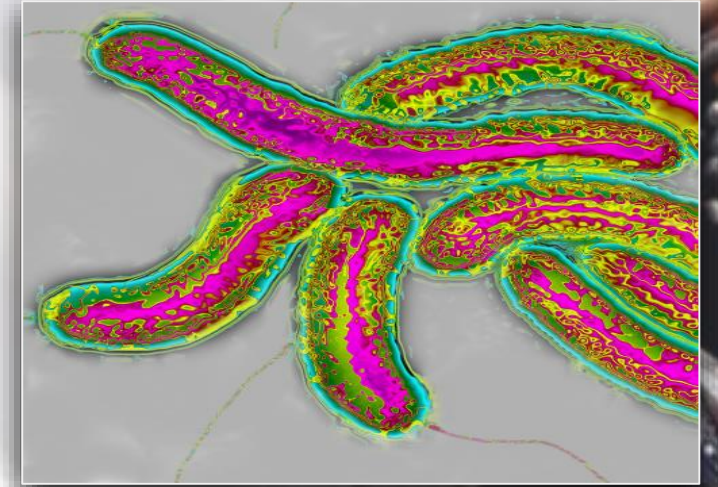
Vibrios

Widespread distribution: Globally vibrios are one of the leading groups of human pathogens responsible for shellfish and waterborne-associated illness. ~ dozen species cause disease in humans.

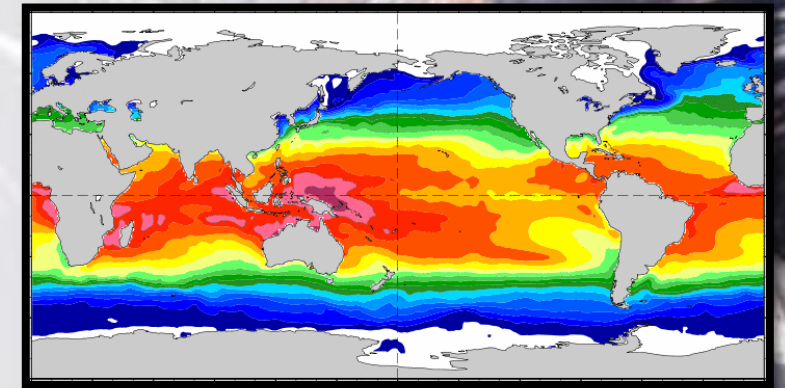
Climate change: These pathogenic bacteria are especially sensitive to climate change – outbreaks are now being observed in temperate areas.

Pandemic potential: Certain strains of vibrios are more pathogenic than others, and capable of rapid pandemic spread.

Changing demographics: Various factors including more at risk/vulnerable groups and greater potential for exposure may increase risks in the future.

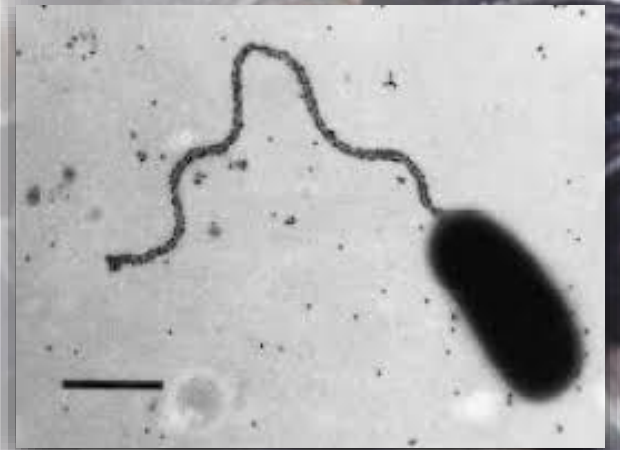


Vibrio parahaemolyticus



Vibrio parahaemolyticus (Vp)

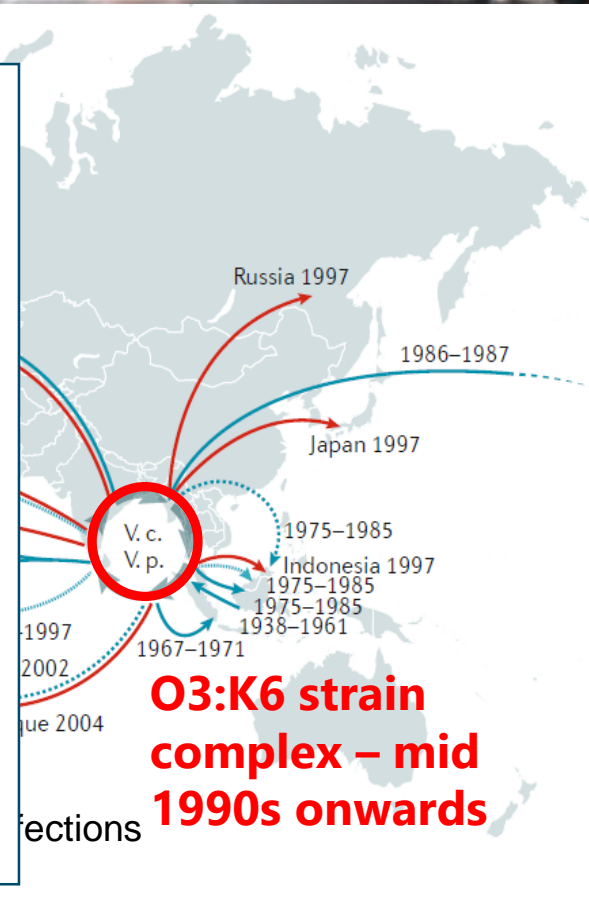
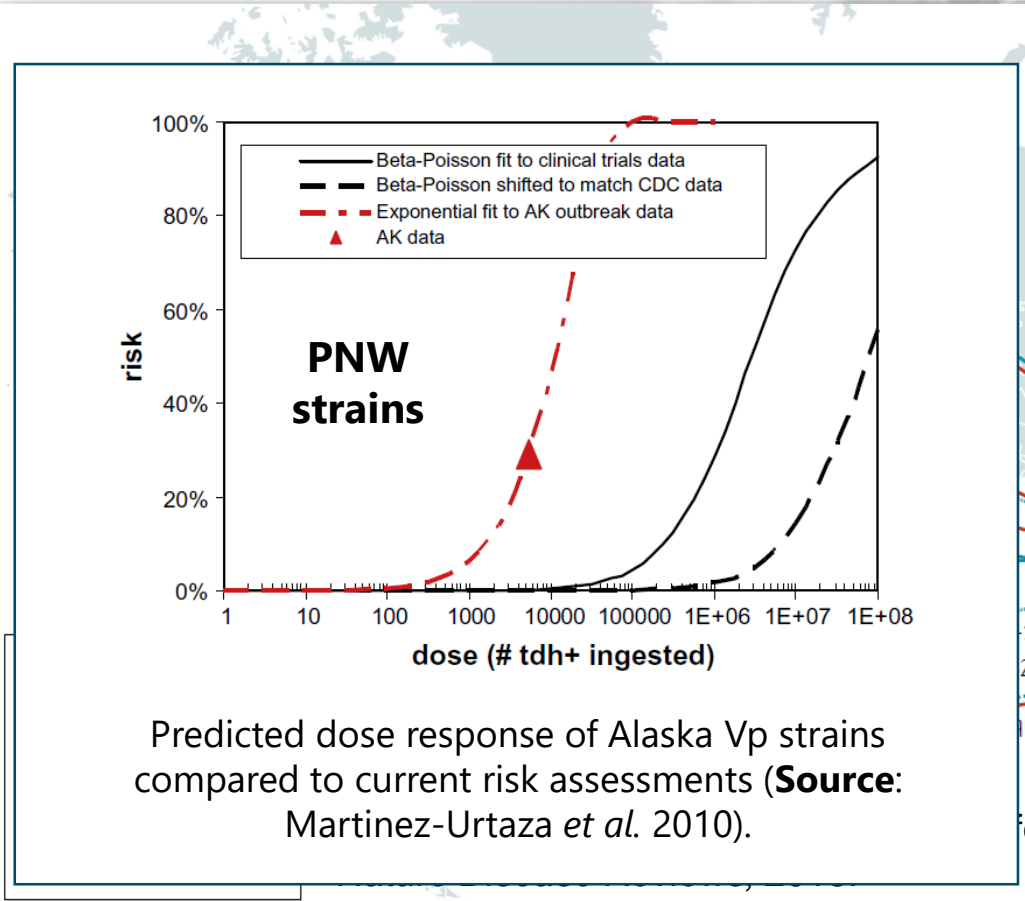
- *Vp* is a common and naturally occurring marine and estuarine bacterium. *Vp* concentrates in bivalve shellfish such as oysters and clams. Globally the most common bacterial infection associated with the consumption of seafood, and often associated with large foodborne outbreaks. We find this pathogen in UK shellfish and water.
- In 2012 a new variant of *Vp* – a highly pathogenic strain normally restricted to Pacific NW region of the USA emerged – jumping to the East Coast of the USA and then Europe. This particular strain (PNW) is of huge concern. Caused major shutdown of US shellfish in 2012 and 2013.
- Pandemic spread of *Vp* is a major global food safety and economic issue.



Pandemic spread of Vp

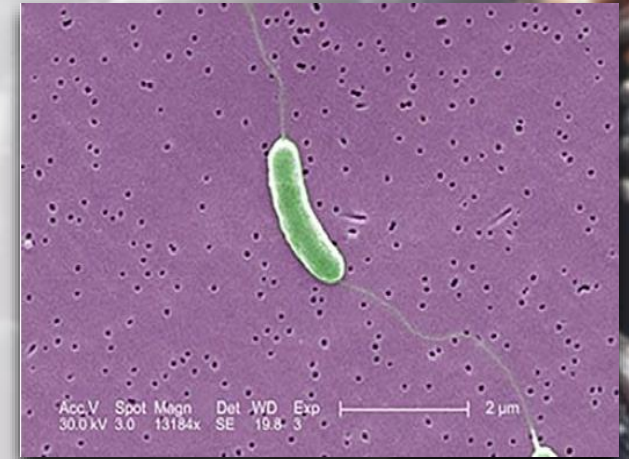
Vp outbreaks are currently undergoing a global process of expansion with infections emerging in areas where these diseases have not been previously observed.

Only pathogen (alongside *V. cholerae* which causes cholera) to spread pandemically from the marine environment. O3:K6 pandemic strain emerged in Asia.



Vibrio vulnificus (Vv)

- Vv is a bacterium common in warm, low salinity marine waters. Found where surface seawater temperatures exceed 15 °C. We now find this bacterium in UK water and shellfish.
- Vv occurs in high numbers in seafood, such as bivalve shellfish, particularly in warmer months - an important opportunistic pathogen that can cause both wound infections and sepsis, typically via exposure to seawater or through consumption of seafood.
- **Vv carries the highest case fatality rate of any foodborne pathogen.** Infections mostly involve males (nearly 90% of cases) the elderly, and at-risk persons. **Increase in reported infections in Europe and USA recently observed. Regularly reported infection in Asia.**



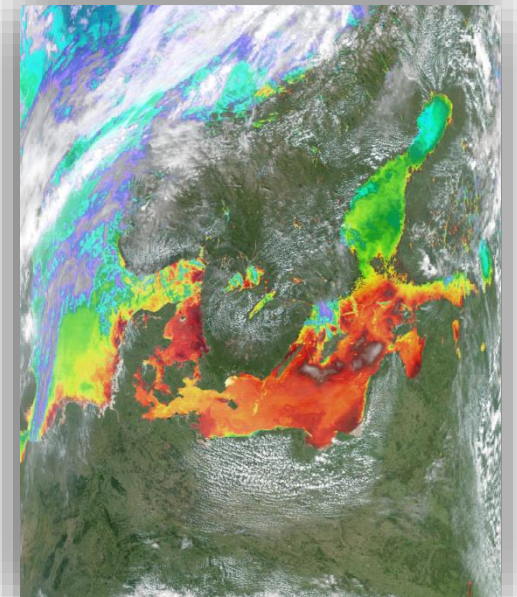
Baker-Austin and Oliver (2019), *Vibrio vulnificus*, *Trends in Microbiology*.



Vibrio vulnificus wound infection. Baker-Austin *et al.* *Nature Disease Reviews*, 2018.

Risk prediction tools: (*Vp*)

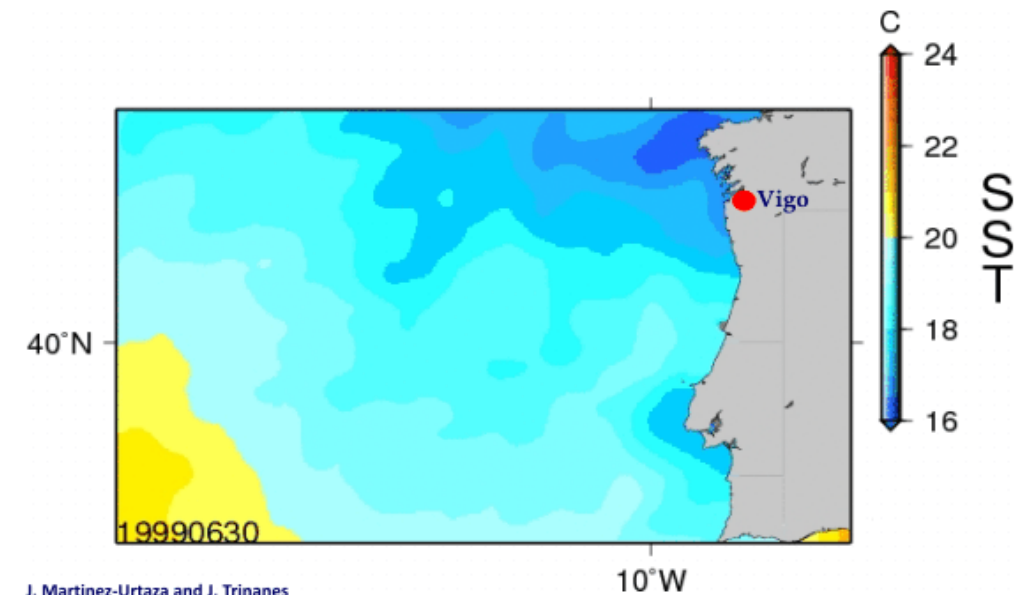
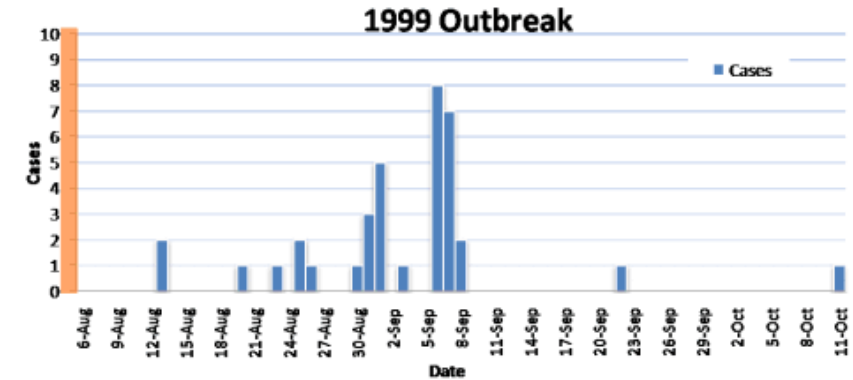
- Remote sensing makes use of satellites to detect infrared radiation emitted from the Earth's surface.
- Several satellites (from Europe and the USA) have been carrying out global studies of sea surface temperature (SST) for the last 3 decades. These datasets allow you to look at any region on the surface of Earth with a high degree of resolution.
- Some case studies presented here that I'll show you suggest that it may be possible to predict *Vibrio* outbreaks using remote data from retrospective studies.
- **We believe that there are often discernible environmental 'signatures' prior to outbreaks.**





Risk prediction tools: (*Vp*)

- During the late summer of 1999, 35 cases of shellfish-associated illness were reported in Northern Spain. All infections caused by *Vibrio parahaemolyticus*. At the time the largest foodborne incident with *Vp* in Europe.
- As you can see - surface seawater temperature appears to play a significant role in when and where these infections were reported.
- One of the 1st times using satellite data has been used to look at the cause of a disease in this way.



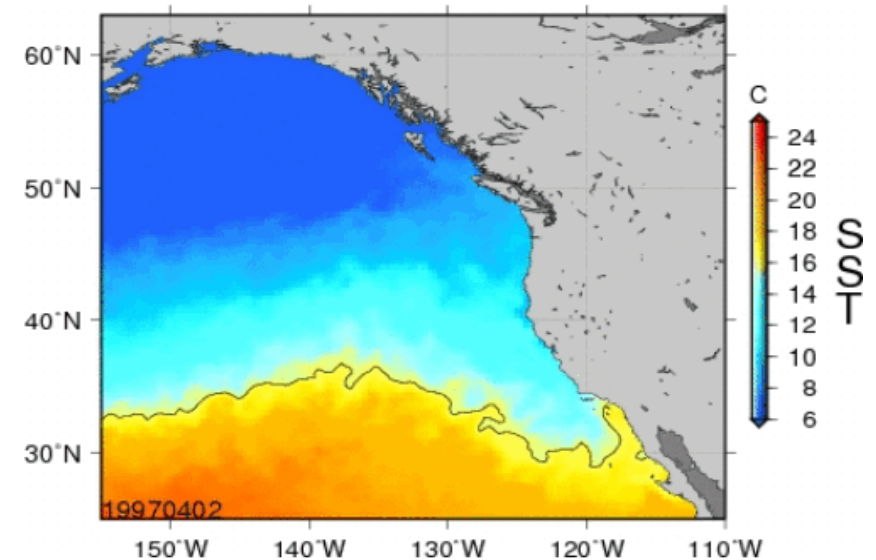
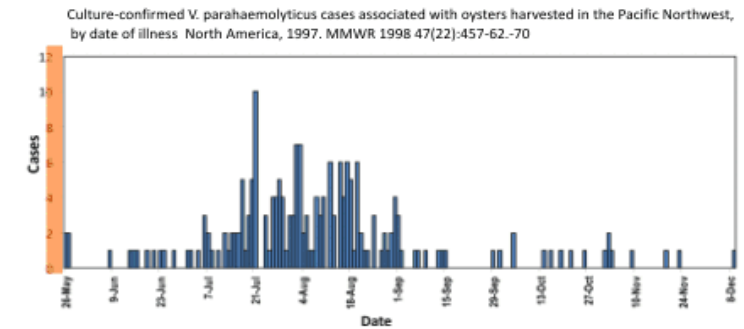
J. Martinez-Urtaza and J. Trinanes
University of Santiago de Compostela, Spain

Source: **Baker-Austin, C.**, L. Stockley, R. Rangdale, and J. Martinez-Urtaza. Environmental occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: a European perspective. *Environmental Microbiology Reports*, 2010.

Risk prediction tools: (*Vp*)

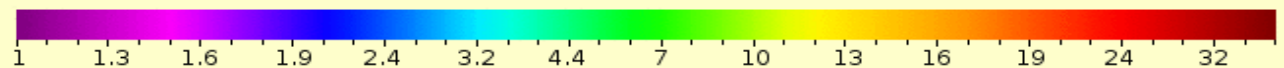
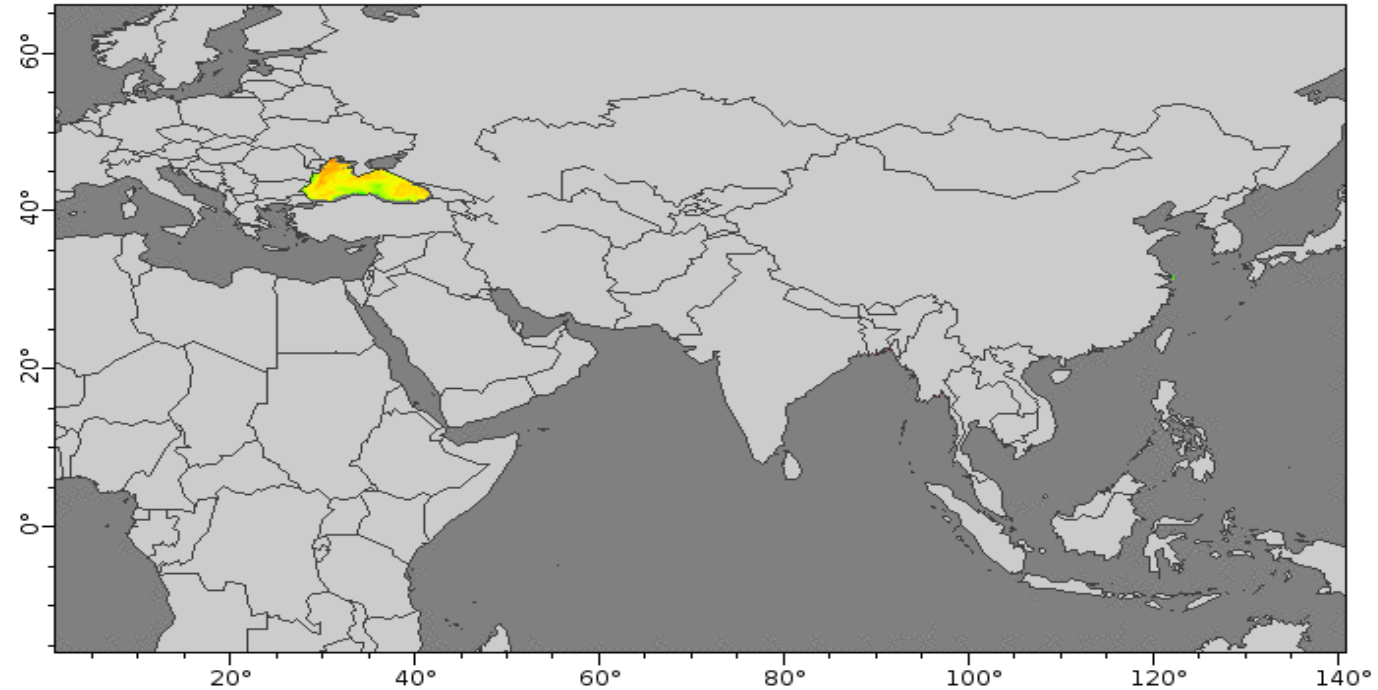


- During the summer of 1997, the largest reported outbreak in North America of culture-confirmed *Vp* infections occurred.
- 209 infections reported, all associated with eating seafood harvested from California, Oregon, and Washington in the United States and from British Columbia (BC) in Canada; one person died.
- Mean Pacific coastal sea surface temperatures were significantly higher (typically 3-5 °C) in areas where cases were subsequently reported.



Risk prediction tools for vibrios

- Consequently, we have helped develop risk prediction tools using remote sensing data.
- Most of these approaches use temperature and salinity data and are updated daily models can also incorporate other variables.
- Extremely useful, and can produce risk-based mapping in near real-time. Some limitations e.g. granularity near coastal regions.



Daily Vibrio Risk (Risk Level)
USDOC/NOAA/NESDIS ECDC USC Vibrio Risk Analysis
(2013-06-12T12:00:00Z)
Data courtesy of CoastWatch Caribb-NOAA AOML ECDC USC/IIT

***Vibrio* risk prediction tool**

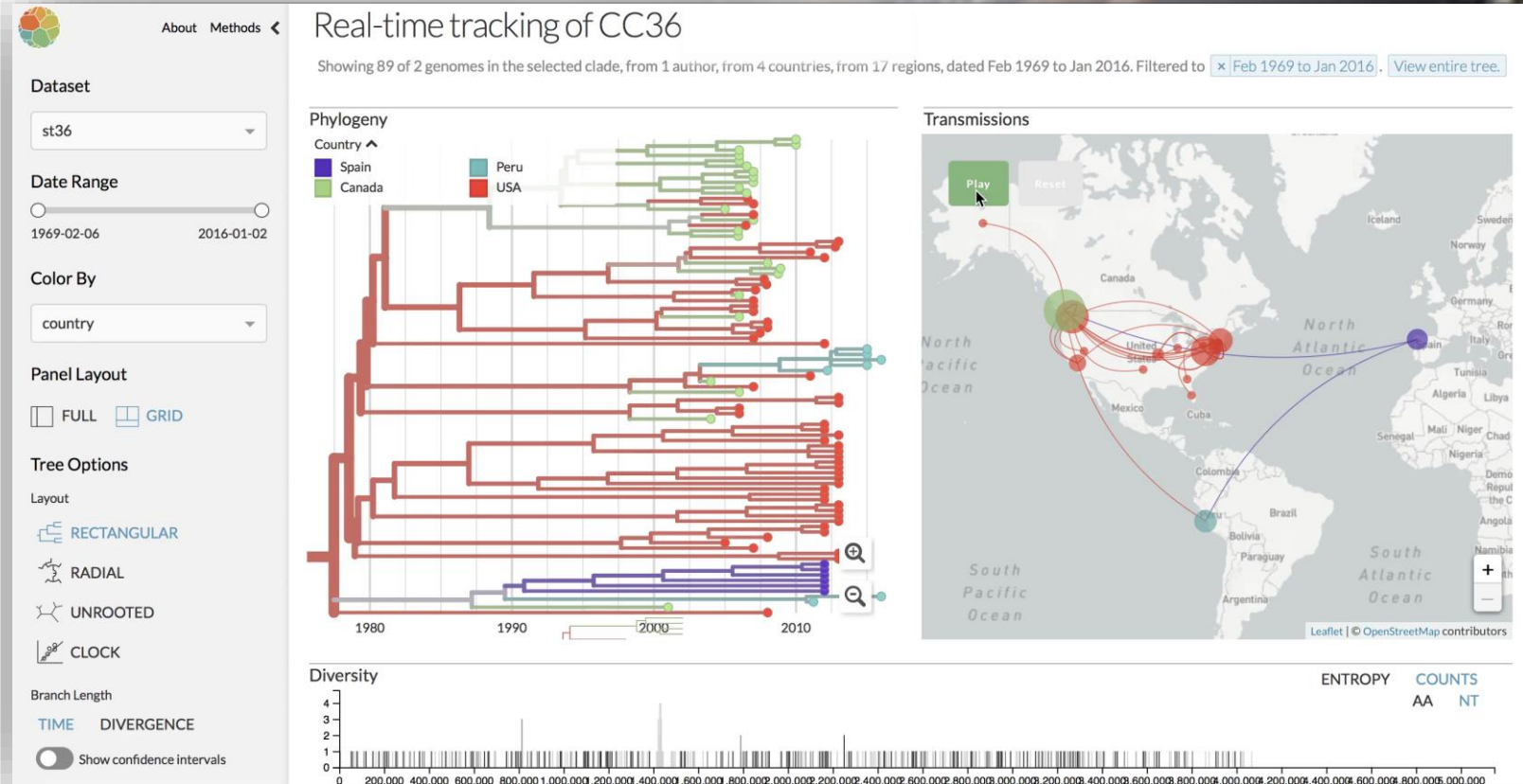
<https://cwcgom.aoml.noaa.gov/cgom/OceanViewer>

High-resolution tools: Genomic analysis of Vp

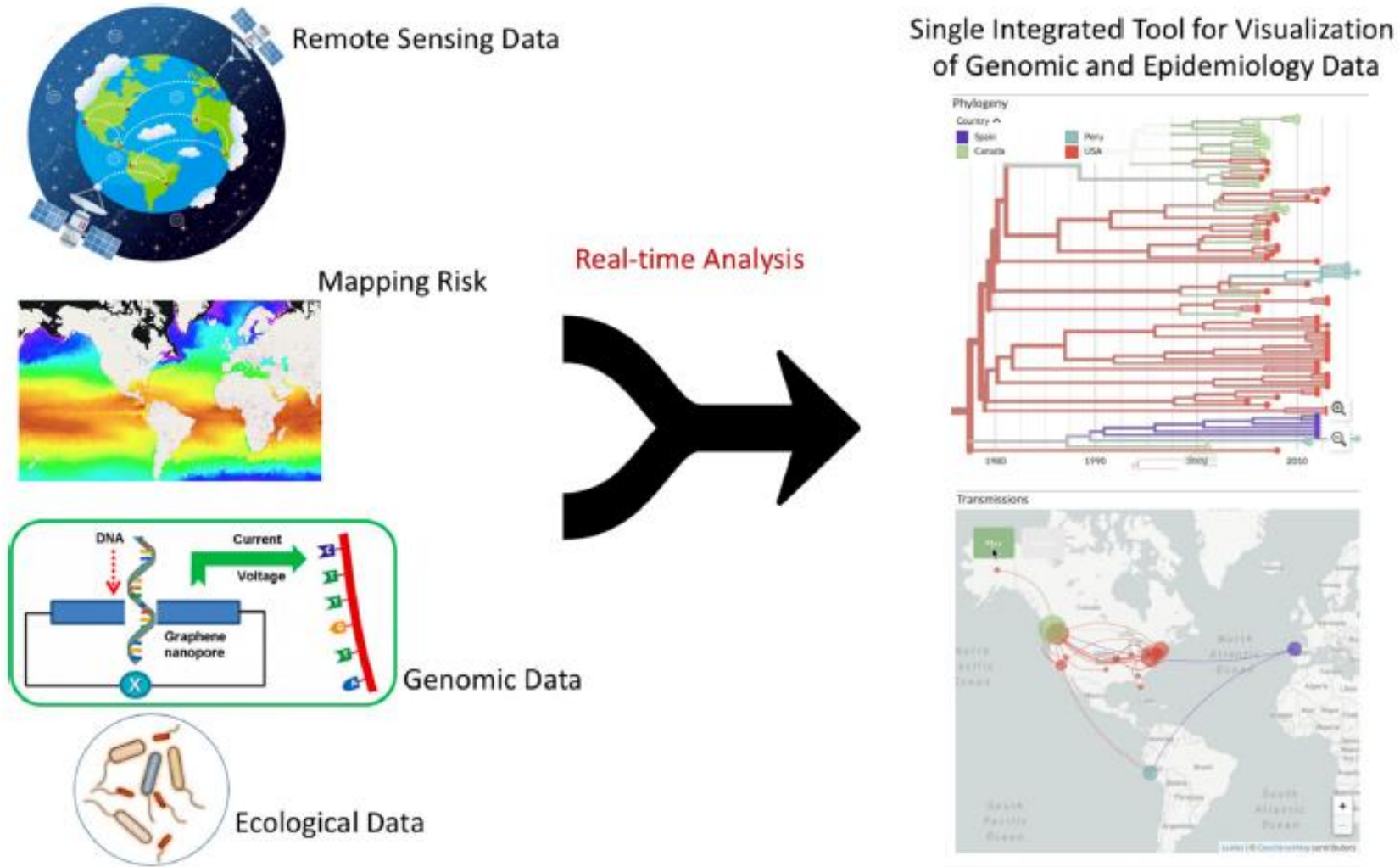
Recent sequencing and bioinformatic approaches can reconstruct the emergence, dispersal, and evolution of microbial pathogens.

The unparalleled resolution of sequencing methods has enormous practical applications such as inferring mechanisms of transmission, unravelling the evolution of strains, as well as pinpointing outbreaks for risk management purposes.

Can be applied to bacteria and viral pathogens e.g. vibrios and NoV, HAV.



Where is the science (and the methods) going?



Source: Baker-Austin, C. *et al.* New tools revolutionising *Vibrio* science, *Environmental Microbiology*, 2020.

Summary

- Globally, norovirus, Hepatitis A and vibrios are the most important human pathogens associated with seafood.
- Dire need for improved systems of epidemiology, surveillance and reporting of shellfish-associated disease – again globally but also in the UK (under-reporting of infections is a very common issue).
- Increased international trade of shellfish, climate change, coupled to demographic and epidemiological changes may likely increase risks.
- Variety of different methods for tackling emerging diseases have been developed in the last decade, including risk prediction tools (e.g. remote sensing, genomics), as well as detection and quantification methods.

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Many thanks for listening

craig.baker-austin@cefas.co.uk

Acknowledgements

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 - Cefas Seedcorn
- Jake Bowley and Dr Ceri Lewis, Dr Stephen Michell University of Exeter
 - Dr Joaquin Tristanes, University of Santiago de Compostela, Spain

